1988

Influence of [alpha]-ketoisocaproate on growth, carcass composition and protein metabolism in cattle and sheep

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Order Number 8825393

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Iowa State University, 1988

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Influence of α-ketoisocaproate on growth, carcass composition and protein metabolism in cattle and sheep

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Animal Nutrition

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1988

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GENERAL INTRODUCTION

Since the origin of life, man has been interested in food for survival, enjoyment and economic benefit. This interest has cultivated curiosity in not only the variety of foodstuffs and their composition, but also in the processes involved in the taking in and utilization of food substances by which growth, repair and maintenance of activities in the body are accomplished. These processes of ingestion, absorbtion and metabolism together are referred to as nutrition. Nutrition has developed as a unique area of science, as daily each person is interested in nutrition for their personal well-being.

Man's curiosity inspired scrutiny into which foodstuff components resulted in improved health, enjoyment and longevity. The "father of medicine," Hippocrates (460-377 B.C.), reflected some of the earliest beliefs on this subject when he stated to the ancient Greeks, "There is but one food, but there exist several forms of food" (Mendel, 1923). Later, the Roman physician Galen (130-200 A.D.) theorized that during digestion all food was changed into a single substance that maintained life. However, with time man dissected foodstuff components into fats, carbohydrates, proteins, vitamins, minerals and water, and it was realized that all nutrients combined in a balanced arrangement were necessary for healthful sustenance.

The French physiologist, Fancois Magendie, was the first to note the need for protein in the diet. In a memoir written in 1816, "Food Substances without Nitrogen," he reported a trial in which dogs fed diets consisting only of sugar, fat and distilled water became emaciated

and died. Magendie concluded that animals cannot live without nitrogenous food (Olmsted, 1944). In 1839, the Dutch chemist Mulder was the first to suggest the term "protein" for the compound that "unquestionably is the most important of all known substances in the organic kingdom. Without it, no life appears possible on our planet" (Mendel, 1923).

Approximately 150 years have passed since these initial observations. During this period, protein and its relationship to bodily functions have been critically researched. Interestingly, with all of the research in this area, the initial premise still withstands: protein is essential for life. Protein and its constituent amino acids are known to be required for many important physiological processes (e.g., hormones and enzymes for control of metabolism, plasma proteins for metabolite transport) and anatomical structures (e.g., muscle tissue, connective tissue, nucleoprotein).

Malnutrition is one of the major problems facing the world today, as it has been estimated that 40% of the world's population is malnourished. Additionally, protein deficiency is considered to be the major contributor to this malnutrition. Kwashiorkor, a disease in which energy is adequate but protein is severely limiting, is widespread throughout the world, particularly where grain is the major dietary foodstuff and protein source. Kwashiorkor and other severe forms of protein malnutrition severely diminish the quality of life, resulting in reduced growth and maturation, in decreased resistance to infective agents and in diminished intellectual and learning capabilities.

With the already existing shortage of quality protein and the foreseen increase in world-wide population, the world-wide supply of protein must be increased. Animal protein production needs to be an important part of this increase. Animal production provides greater than half of the world's protein (CAST, 1975). In addition to quality protein, animal products provide one-fourth of the energy consumed, four-fifths of the calcium consumed, two-thirds of the phosphorus consumed, significant amounts of other minerals and vitamins (especially vitamin B_{12}) and a variety of important byproducts.

Ruminants are particularly unique in that they can convert non-protein nitrogen and low-quality protein to a high-quality foodstuff for human consumption. Forages, which are poorly digested by humans, contribute 70% of the protein consumed by dairy cattle, 60% of the protein consumed by growing beef cattle and 85% of the protein consumed by growing lambs (Moore et al., 1967). The beef industry has been estimated to provide Americans with 12% of their total food-energy intake and 23% of their total protein intake, the highest of any agricultural commodity (CAST, 1979).

Not only will the world need to increase the production of protein, but an increase in the efficiency of this production is also necessary. An improvement in efficiency, with an equal number of inputs, would naturally increase the amount of protein produced. However, an improvement in efficiency may also be a mechanism to allow the agricultural community to maintain or increase a margin of profit by requiring fewer inputs. Finally, if society and policy-makers decide

that more grain needs to be directed to human consumption, there would be less available for livestock and livestock would be required to be more efficient to compete.

The efficiencies at which animals produce food protein are included in Table 1. Although it should be remembered while interpreting these data that much of the ruminant feed comes from sources poorly utilized by humans, it is clear that there is considerable margin and need for improvement in efficiency.

Table 1. Efficiency of various livestock in converting feedstuffs to edible nutrients[a]

Livestock	-Efficiency of nutrient conversion, %-[b] Crude protein	Energy
Nonruminants:		
Broiler	23	11
Turkeys	22	9
Hens (eggs)	26	18
Swine	14	14
Ruminants:		
Dairy cattle	25	17
Beef cattle	4	3
Lambs	4	3

[a]Summarized from Wedin et al. (1975).

[b]Efficiency of nutrient conversion is equal to the amount of nutrient consumed divided by the amount of nutrient produced multiplied by 100%.

In animals used for production of protein, energy is expended for various biological functions. While a certain amount of protein degradation is necessary for normal biological function, unnecessary protein turnover would result in nutrient waste. Therefore, decreasing protein turnover is a possible method to improve animal protein production. Decreased protein turnover would result in less energy being required to produce a kg of meat, milk or egg protein. The object of the following research is to examine the effect of α-ketoisocaproate, which has been proposed to reduce protein turnover, on growth, efficiency of feed conversion, muscle and fat deposition and protein metabolism in growing cattle and lambs.

REVIEW OF THE LITERATURE

General Leucine and KIC Metabolism

Biochemical pathways and enzymes Leucine is a 6-carbon amino acid ($(CH_3)_2CHCH_2CH(NH_2)COOH$) that is essential to mammalian tissues as it cannot be synthesized de novo within the body. It belongs to the class of amino acids called branched-chain amino acids (BCAA) of which isoleucine, valine and leucine are the only members. The major difference between the metabolism of the three amino acids is the products produced. Whereas leucine is ketogenic, valine is glucogenic and isoleucine is both ketogenic and glucogenic.

Leucine in the body is derived from three sources: protein breakdown, dietary leucine and transamination from the α-ketoacid, α-ketoisocaproate (KIC). Leucine either can be incorporated into protein or transaminated to KIC. Leucine is converted to KIC via the enzyme BCAA transaminase, which is specific for all BCAAs, and leucine transaminase, which is specific for leucine only. This transamination is reversible, and KIC can be converted back to leucine.

KIC is irreversibly oxidized to isovaleryl CoA via the mitochondrial enzyme branched-chain keto acid (BCKA) dehydrogenase. The activity of this enzyme is stimulated by increased dietary protein, increased dietary leucine or KIC and diabetes. Phosphorylation inactivates the enzyme. In addition to the conversion of KIC to isovaleryl CoA, KIC can be decarboxylated to β-hydroxy-β-methylbutyrate (HMB) in the cytosol by KIC oxygenase. It is thought that HMB is

excreted in the urine.

Isovaleryl CoA is sequentially converted to β-methylcrotonyl CoA, β-methylglutaconyl CoA and finally β-hydroxy-β-methylglutaryl CoA (HMG CoA) within the mitochodria. HMG cleavage enzyme metabolizes HMG CoA to acetoacetate plus acetyl CoA. Acetoacetate can cross the mitochondrial membrane and be converted to ketone bodies (acetone and β-hydroxybutyrate) or be sequentially converted to acetoacetyl CoA and HMG CoA. In the cytosol, HMG CoA can be metabolized to mevalonic acid via HMG CoA reductase. Mevalonic acid continues on to cholesterol and other steroid synthesis. Therefore, the end products of leucine and KIC metabolism include carbon dioxide, ketone bodies, HMB and cholesterol.

Physiology of BCAA and BCKA metabolism The distribution of the enzymes involved in BCAA metabolism varies with different body tissues. BCAA transaminase was not detected in rat liver but was detected in the brain, muscle and kidney (Khatra et al., 1977). Mimura et al. (1968) noted BCAA transaminase activity in rat small intestine. When KIC was added to kidney perfusate, leucine was released at a rate amounting to 15 to 25% of the total KIC disappearance (Mitch and Chan, 1978). Interestingly, KIC, but not leucine and isovaleryl Co A, addition increased the rate of transamination. It was suggested that KIC may control transamination activity, therefore regulating the metabolism of BCAA (Mitch and Chan, 1979).

In the rat, 70% of the total BCKA dehydrogenase activity is found in the liver and 10% in the muscle (Bassler and Pietrek, 1983). In man, the liver accounts for 30% of the total BCKA dehydrogenase activity

whereas muscle tissue accounts for 60% of the activity (Khatra et al., 1977). Therefore, the conditions for an oral dose of KIC passing through the liver is more favorable in man than in the rat.

These differences in where the enzymes are found result in important differences in the site of metabolism between BCAA versus BCKA. It is thought that BCAA undergo very little metabolism in the liver and gut tissues, but rather are metabolized mainly by muscle and somewhat by adipose tissues. Miller (1961) first proposed that a significant amount of BCAA was metabolized by extrasplanchnic tissues. Early canine liver perfusion studies indicated that only 15% of a BCAA load was taken up by the liver (McMenamy et al., 1962). In vivo studies in unanesthetized dogs confirmed that most BCAA (~70%) escape the liver and are taken up by extrasplanchnic tissues after the ingestion of a protein meal (Elwyn et al., 1968). In another study with dogs, Bloomgarden et al. (1981) found that 95% of the BCAA escaped the liver and were taken up by the extrasplanchnic tissues (Bloomgarden et al., 1981). It was noted in humans that most of the BCAA were taken up by extrasplanchic tissues after the ingestion of a protein meal (Aoki et al., 1976; Wahren et al., 1976). In a study with lambs, dietary leucine was absorbed at the rate of 1.1 mmol/hr and the liver removed .4 mmol/hr (33% removed; Wolff et al., 1973).

In studies with isolated rat muscle (Chang and Goldberg, 1978b) and perfused rat muscle (Hutson et al., 1979), it was concluded that skeletal and heart muscle were the major if not the only site for metabolism of BCAA. Abumrad et al. (1982a) intravenously infused amino

acids into the human forearm and measured the balance of amino acids across this site. Although 35% of the amino acid solution infused was BCAA, BCAA accounted for 49% of the total amino acid uptake of the forearm. They concluded that, while skeletal muscle is an important site for the uptake of BCAA, it cannot account for more than 65% of the load administered. These data, across a variety of species, similarly emphasize the minimal role of the liver and the major role of the muscle to metabolize BCAA.

Although muscle tissue is thought to be the primary contributor to leucine metabolism, adipose tissue also has the ability to be very active in the metabolism of leucine (Rosenthal et al., 1974; Wijayasinghe et al., 1983).

In direct contrast to leucine and BCAA, KIC and BCKA have been implicated to undergo extensive catabolism in splanchnic tissues. As noted earlier, there is considerable BCAA transaminase activity in intestinal and hepatic tissues (Bassler and Pietrek, 1983). Weber et al. (1977) infused ketoacids into the jejunum of fasting dogs to measure transport across the gut and found 20 umol KIC/(cm*hr) disappeared from the lumen of the jejunum. Of the total KIC absorbed, 8% appeared in mesenteric blood as leucine and 34% of the absorbed KIC was degraded by the gut wall. Similar studies utilizing rat jejunum and iliac segments of intestine also reported significant release of infused KIC as leucine, indicating partial transamination of KIC in the gut wall (Weber et al., 1979).

Abumrad et al. (1982b) extensively examined the site of KIC

metabolism after administering an oral load of KIC to dogs. They found that after about one hour approximately 63% of the KIC load was absorbed as KIC, while 23% was transaminated across the gut and released as leucine. The liver removed 35% of the administered load, of which 33% was transaminated to leucine and 66% was converted to ketone bodies. Therefore, 27% of the administered load passed through the gut and liver tissues as unmetabolized KIC. Of this, approximately 12% was transaminated across the kidneys to leucine, leaving only 15% of the original dose for use by peripheral tissues. No mention was made as to uptake via muscle versus other peripheral tissues such as adipose or brain.

Similar results were obtained with the rat, where only 12% of an oral load of KIC passed through the gut and liver unchanged (Bassler and Pietrek, 1983). KIC was not detected in feces from these animals.

KIC has been shown to be rapidly catabolized by muscle. In early studies in liverless dogs where 1 g of KIC was infused intravenously, there was no increase in the arterial plasma, suggesting a very rapid uptake (McMenamy et al., 1965). The half-life of an intravenous dose (1 mmol) of KIC was found to be 10.5 min (Bassler and Pietrek, 1983). Intra-arterial infusion of KIC (34.3 umol/min) into the human forearm resulted in 52% of KIC extracted in a single passage of KIC (Pozefsky and Walser, 1977). Leucine was the only amino acid released in increased amounts and accounted for 31% of the KIC extracted. Alanine, histidine and glycine release were significantly lowered, indicating that these amino acids may be the nitrogen donors for the conversion of KIC

to leucine.

In humans and rats, starvation increases plasma BCAA concentrations, whereas protein deprivation decreases plasma BCAA concentrations within one day. Changes in the plasma concentrations reflect changes predominantly in muscle and secondly in the liver. The increased muscle protein breakdown during starvation, which supplies substrates for enhanced gluconeogenesis in the liver and enhanced BCAA oxidation in the muscle, is the major mechanism of hyperbranched-chain aminoacidemia during starvation. The main factors in the development of hypobranched-chain aminoacidemia during protein deprivation are absence of exogenous amino acids as well as curtailed protein breakdown (Adibi, 1976).

Leucine and KIC metabolism were measured in lambs fed either high-energy or low-energy diets (Nissen and Ostaszewski, 1985). Less than 20% of the total leucine was transaminated to KIC and 42% of the KIC was converted to leucine, indicating that transamination is low in ruminants. Increased dietary energy decreased leucine incorporated into protein, leucine oxidation and interconversion of KIC and leucine.

Starvation in lambs produced a decrease in plasma BCAA and BCKA (Bergman and Heitman, 1978; Pell et al., 1986). Unlike humans and rats where BCAA from proteolysis during starvation are catabolized locally, lambs fasted 3 to 6 days released considerable BCAA from the hind quarter muscles. During the fast, KIC turnover increased while leucine turnover decreased. KIC flux was approximately 10% that of leucine. About 40% of KIC and 15% of leucine were oxidized, but they contributed

less than 1% of the whole-body CO_2 production. Portal-drained visera released KIC and leucine into the periferal blood only in the fed sheep. Liver utilized 2 and 12 umol/min of KIC and leucine during both the fed and fasted states. Muscle produced leucine and KIC during fasting and utilized both during fed states. After fasting leucine going to whole-body protein synthesis decreased 27%. Hepatic protein synthesis was maintained at the expense of muscle during fasting.

These experiments pointed out several differences in ruminant and nonruminant BCAA and BCKA metabolism. First, during fasting the ruminant liver utilizes leucine, whereas the nonruminant liver produces leucine. In addition, muscles of the hindquarters produce leucine in ruminants, whereas nonruminants appear to oxidize leucine in muscle. Finally, although the interconversion of KIC and leucine is extensive in nonruminants, it is limiting in ruminants.

Leucine and KIC Effects on Protein Metabolism

In vitro protein turnover studies

Leucine The first report proposing leucine as a regulator of protein metabolism was made in 1971 by Morgan et al. They found that in vitro synthesis of whole-heart protein and myosin was increased by 40% when amino acid concentrations were increased from 1 to 5 times normal plasma concentrations. Odessy et al. (1974) later found that BCAA inhibited net protein synthesis while increasing the de novo synthesis of alanine and glutamine, which are energy substrates for liver, kidney and gut. Another study in the same laboratory reported

that leucine increased protein synthesis and decreased proteolysis in muscle under in vitro conditions (Fulks et al., 1975).

Buse and Reid (1975) also proposed leucine to be a regulator of protein turnover as they discovered leucine, but not valine or isoleucine, increased protein synthesis in muscle. This effect was not blocked by dactinomycin, indicating that the stimulation was at the level of previously formed messenger RNA. Furthermore, the injection of leucine into fasted rats treated with glucose and insulin increased the proportion of polysomes to subunits in ribosomal preparations of psoas muscles (Buse et al., 1979). The effect on protein synthesis was therefore proposed to be exerted at the level of translation and appears to involve peptide chain elongation. Later reports from the same laboratory again utilizing rats indicated that BCAA doubled protein synthesis in diaphragm muscle, promoted lesser increases in soleus and psoas muscle-protein synthesis and caused no significant increase in gastrocnemius and heart muscle-protein synthesis (Buse, 1979).

Lundholm et al. (1981), using the rectus abdominus muscle from humans and the extensor digitorum longus muscle from rats, demonstrated that the addition of leucine to the incubation media decreased the rate of protein degradation. This was further decreased by the addition of other amino acids. In these experiments, insulin increased protein synthesis but did not alter protein degradation.

Tischler and Goldberg (1980a) found that leucine affects protein breakdown in skeletal and cardiac muscle but not in adipose tissue. They proposed that the inhibitory effects may be mediated by changes in

oxidation-reduction state (Tischler, 1980). Additionally, it has been suggested that exogenous BCAA may act as an energy source in muscle and slow down amino acid efflux (Freund et al., 1980).

L-norleucine, L-norvaline, D-norleucine and L-allo-isoleucine were found to be as effective as leucine in inhibiting protein degradation, but none of these had an effect on protein synthesis (Grinde and Seglen, 1981). In this study, KIC did not significantly alter proteolysis.

KIC Although leucine appeared to have a unique role in the regulation of protein metabolism, two separate groups postulated that it was not leucine, but one of its metabolites, namely KIC, that was the active controller of protein metabolism. Chua et al. (1979) were the first researchers to suggest that KIC had effects on protein turnover. These researchers noted that in rat heart muscle leucine, but not isoleucine or valine, inhibited protein degradation and accelerated protein synthesis. KIC also decreased proteolysis and increased protein synthesis without increasing the intracellular concentration of leucine, whereas the keto-acids of isoleucine and valine had no effect on protein turnover. Isovalerate also decreased proteolysis and increased protein synthesis. Therefore, it was suggested that, although leucine has regulatory effects on protein metabolism, its metabolites also possess some of these same regulatory effects.

In perfused rat livers, Poso et al. (1982) found that leucine, tyrosine, glutamine, proline, histidine, tryptophan and methionine suppressed proteolysis, with leucine being the most potent inhibitor.

Leucine decreased proteolysis by 63% when added at 4 to 10 times the concentrations normally found in plasma. However, at concentrations normally found in plasma, leucine did not decrease proteolysis, whereas tyrosine, glutamine, proline, histidine, tryptophan and methionine continued to depress proteolysis at normal concentrations. The fact that leucine has effects on proteolysis at concentrations higher than normal, may have physiological significance because the liver and peripheral tissues are exposed to high leucine concentrations after feeding when increased protein synthesis and reduced proteolysis are needed for the deposition of the absorbed amino acids. KIC produced responses similar to leucine while increasing intracellular leucine by only 7.8%. These researchers noted that, when KIC was added at 4 to 10 fold times the concentrations normally found in plasma, transamination limits interconversion between between leucine and KIC to 4 to 10% of total KIC utilization. Additionally, decarboxylation was demonstrated to be 10 fold higher than transamination. They concluded that the effects of leucine and KIC on hepatic proteolysis are mediated by separate pathways.

Rat diaphragm muscles were found to have increased protein synthesis with the addition of .1 mM of leucine (Tischler et al., 1982). Protein degradation was not altered until .2 to .5 mM of leucine were added. These effects were found in the fed or fasted state, unlike leucine inhibition of glucose oxidation which is specific to the fasted state (Tischler and Goldberg, 1980a). When leucine transamination was inhibited with L-cycloserine, leucine no longer inhibited proteolysis.

Additionally, KIC at .5 mM decreased proteolysis without increasing protein synthesis. From these three studies, it can be concluded that KIC primarily alters protein turnover by lowering proteolysis.

Although it is debatable as to whether KIC increases muscle protein synthesis, KIC has been shown to increase the synthesis of other proteins. The rate of albumin synthesis was increased when KIC was added to blood perfusing rat livers (Kirsch et al., 1976). In addition, a mixture of BCKA increased albumin synthesis per liver weight levels when administered intragastrically to rats 16 hr after hepatectomy.

Other examples of BCAA and BCKA altering protein synthesis have been reported. Leucine was shown to regulate the secretion of prolactin in vitro and increase the release of prolactin from pituitary mammotrophs (Cross and Walker, 1984; Miller et al., 1986). A 9-hr infusion of BCAA increased the peak frequency of growth hormone secretion in baboons (Stewart et al., 1984). KIC has been shown to increase insulin release (Panten et al., 1974; Holze and Panten, 1979) and proinsulin biosynthesis (Hutton et al., 1980).

In vivo nitrogen balance

Leucine Under normal conditions, the liver extensively catabolizes aromatic amino acids, methionine and histidine. However, the liver contributes very little to the catabolism of BCAA. Therefore, under normal conditions, BCAA make up 60 to 100% of the amino acids that clear the liver (Felig, 1980). However, during chronic liver disease with acute exacerbation, aromatic amino acids, methionine, aspartate and

glutamate are increased above normal concentrations, whereas BCAA concentrations are reduced by 50 to 60% (Sax et al., 1986).

It would stand to reason, therefore, that the first proposed clinical use of BCAA was in the treatment of chronic liver disease (Fischer et al., 1975). During liver failure, increased amino acid flow through the liver results in these amino acids accumulating at nerve endings, replacing the normal transmitters both within the brain and peripheral nervous system, which results in nervous system abnormalities, encephalopathy and hepatic coma (Fischer and Baldessarini, 1971). Additionally, hepatic failure is hypercatabolic. Several reports have been published in which BCAA improved liver disease and hepatic encephalopathy (Fischer et al., 1976; Higashi et al., 1981; Okada et al., 1981; Freund et al., 1982) by providing an energy source, decreasing muscle breakdown, increasing hepatic protein synthesis, normalizing the plasma and brain amino acid patterns, normalizing catecholamine levels and increasing the metabolism of ammonia in skeletal muscle (Sax et al., 1986).

BCAA have also been shown to be useful under conditions of sepsis, which is the single highest remaining cause of mortality in surgical patients. Sepsis is the pathological state resulting from toxins usually produced by microorganisms. During sepsis, muscle breakdown is increased as more amino acids are required for energy and synthesis of host defense proteins, excessive urea is produced, the liver and other organs fail and with this failure the amino acid balance is again altered. BCAA treatment would tend to help correct these symptoms (Sax

et al., 1986).

Experiments examining other stressful situations have been less definitive. There are various problems associated with many of these studies including differing levels of stress within an experiment and lack of true controls. To briefly summarize these experiments, however, BCAA improved nitrogen balance and recovery in several experiments but had no effect in others.

BCAA, administered enterally through a needle-catheter jejunostomy to 20 patients, were found to produce positive nitrogen balance within 2 or 3 days after abdominal surgery (Andrassy et al., 1985). However, there were no true controls in this study. Infusion of BCAA to fasted and septic rats reduced whole-body nitrogen loss and spared liver protein by increasing the fractional synthesis of the liver and muscle proteins (Blackburn et al., 1979). Holecek et al. (1985) performed a partial (65 to 70%) hepatectomy on rats after which they administered either a control solution of amino acids or the same solution plus extra BCAA. The onset of DNA synthesis was more rapid in animals fed the enriched solutions. When Rigotti et al. (1985) infused rats with portalcaval shunts with BCAA, survival was lengthened and neurological function was preserved longer. Additionally, BCAA lowered plasma and brain ammonia concentrations, while increasing alanine and glutamine concentrations. Ten malnourished cancer patients were provided with isonitrogenous amounts of either a conventional total parenteral nutrition diet containing 19% BCAA or a branched-chain amino acid enriched diet containing 50% of the amino acids as BCAA (Tayek et al.,

1986). The BCAA enriched diet increased whole-body leucine flux, oxidation and protein synthesis. Protein breakdown and urinary nitrogen loss was not altered.

Severe burns result in highly catabolic states. Initially after a burn, there are decreased levels of all amino acids, but in particular, BCAA are decreased (Stinnett et al., 1982). However, Snelling et al. (1982) did not note a preference for peripheral use of BCAA over phenylalanine. Additionally, a 45% BCAA solution did not significantly increase nitrogen balance in severely burned rats (Sax et al., 1986).

Another disease associated with considerable wasting of muscle protein is muscular dystrophy. Hudecki et al. (1982) found that twice-daily injections of BCAA increased righting ability measured by standardized flip-test procedure of chickens with muscular dystrophy. However, Mendell and co-workers (1982) administered leucine to Duchenne muscular dystrophy patients for several months and reported no improvements over controls.

KIC Richards et al. (1967) were the first to suggest the use of KIC in a clinical setting. They theorized that ketoacids of essential amino acids may be useful in the treatment of uremia by reutilizing urea nitrogen for conversion of ketoacids to amino acids, lowering blood urea nitrogen levels and increasing protein synthesis. Commercial availability of ketoacid compounds limited research of this theory.

The first study using KIC for treatment of uremia was conducted several years later by Walser et al. (1973). Ten patients with severe

chronic uremia were fed a diet low in protein and sufficient in calories plus 6 to 14 g KIC daily for 15-18 days. When KIC-treatment was discontinued, plasma urea-nitrogen appearance increased and nitrogen balance decreased. Kampf et al. (1980), Horl et al. (1980) and Attman et al. (1984) demonstrated improvements in uremic conditions when KIC was administered. Walser et al. (1983) listed several mechanisms that explain why ketoacids may be used in treating uremia: 1) permitting a decrease in nitrogen intake, 2) decreasing protein breakdown and 3) increasing protein synthesis.

Several studies were conducted in which KIC was administered to patients with severe chronic renal failure. Ell et al. (1978) treated 5 patients with KIC and reported a decrease in plasma urea concentration, urea synthesis and urea excretion plus an improvement in nitrogen balance. Albumin synthesis was also increased. They concluded that this improvement in nitrogen balance could not be accounted for solely by the capture of nonprotein nitrogen by the exogenous KIC. Alverstrand et al. (1980), Frohling et al. (1980) and Kampf et al. (1980) confirmed these results by demonstrating increased survival rate and prolonged periods of conservative treatments before kidney transplant was necessary. Conversely, Hecking et al. (1980) reported no effect on nitrogen metabolism when patients with chronic renal failure were treated with KIC.

When fasting obese subjects were given parenteral infusions of BCKA daily for one week, urinary urea and total nitrogen excretion were lowered (Sapir and Walser, 1977). This improvement continued up to 17

days after ketoacid infusion was terminated. Plasma BCAA were increased, whereas other plasma amino acids were decreased by ketoacid treatment. Ketone bodies, free fatty acid, glucose, insulin or glucagon concentrations were not affected by treatment. In another study with fasted obese men, Mitch et al. (1981) demonstrated that perenteral BCKA spared whole-body nitrogen and enhanced muscle bulk.

The effects of KIC and leucine on nitrogen metabolism in postoperative patients were described by Sapir et al. (1983). After major abdominal surgery was performed on 21 patients, the only source of calories received were 10 g of glucose, 70 mmol of leucine or 70 mmol of KIC daily (all isocaloric). Whereas leucine had no effect on nitrogen balance, 3-methylhistidine excretion and concentrations of pre-albumin and pre-retinol-binding protein in plasma, concentrations of acetoacetate in plasma were increased. KIC-treated patients had improved nitrogen balance, lowered 3-methylhistidine excretion and increased plasma pre-albumin, pre-retinol-binding protein and ketone bodies. They concluded that KIC, but not leucine, diminishes nitrogen wastage in postoperative patients possibly through increased ketosis, reduced protein degradation or an effect on liver protein.

When KIC was administered twice-daily to chickens with muscular dystrophy, righting ability was improved, pectoralis major muscle mass and protein content was increased and the number of degenerating muscle fibers were decreased (Hudecki et al., 1982). In a separate trial, patients with Duchenne muscular dystrophy were administered KIC orally for four days. During this short experiment,

protein breakdown was decreased.

In addition to effects on nitrogen metabolism in these clinical trials, KIC was found to also alter phosphorus and calcium metabolism. When KIC and other keto acids were administered to patients with chronic renal failure or uremia, it was noted that plasma phosphate concentrations were decreased (Walser, 1975; Heidland et al., 1978; Frohling et al., 1980; Hecking et al., 1980; Kampf et al., 1980; Attman et al., 1984). One explanation given for this decrease was that it was a result of the anabolic effect of ketoacid treatment. Heidland et al. (1978) postulated that the reduced serum phosphate was the result of decreased phosphate intake or increased fecal phosphate excretion due to formation of calcium-phosphate complexes in the gut. Another explanation for this alteration of phosphorus metabolism may involve the reduction in parathyroid hormone concentrations previously reported with ketoacid therapy (DiLandro et al., 1982; Schmicker et al., 1982; Lindenau et al., 1982; Barsotti et al., 1982). Although most of these studies reported no change in blood calcium, there was one report of hypercalcemia due to keto acid infusion (Attman et al., 1984). This observation was attributed to increased calcium intake with calcium $(KIC)_2$ treatment.

Effects of KIC on Carbohydrate Metabolism

Although KIC is considered to be ketogenic, it has been shown to interact with carbohydrate metabolism and spare glucose. Chang and Goldberg (1978a,b,c; Goldberg and Chang, 1978) reported that

leucine provides a significant source of energy to muscle and reduces the oxidation of glucose in fasted animals. Infusions of leucine in the human forearm have been demonstrated to decrease glucose oxidation, resulting in a sparing of glucose (Abumrad et al., 1982a). KIC also has been proposed to decrease pyruvate utilization and spare glucose (Chang and Goldberg, 1978c; Tischler and Goldberg, 1980a).

Effects of KIC on Lipid Metabolism

The pathways of KIC catabolism directly cross the pathways of lipid metabolism as KIC can be converted to ketone bodies or cholesterol. In 1944, Bloch noted that deuterium-labeled leucine was converted to plasma cholesterol. Leucine and KIC are obligatorily catabolized to the cholesterol precursor β-hydroxy-β-methylglutaryl CoA (HMG CoA). However, production of HMG from KIC is thought to be a mitochondrial process, whereas cholesterol synthesis is a cytosolic process. The most likely mechanism whereby KIC is converted to cytosolic HMG starts with the intramitochondrial oxidation of KIC to HMG CoA, followed by its cleavage via HMG CoA lyase to acetoacetate and acetyl CoA. Acetoacetate can enter the cytosol and be converted back to HMG CoA via HMG CoA synthetase (Mathias et al., 1981). HMG CoA can be enzymatically deacylated to HMG (Dekker et al., 1958), which is a known competitive inhibitor of HMG CoA reductase, a major regulatory enzyme for cholesterol synthesis (Fimognari and Rodwell, 1965; Beg and Lupien, 1972). Several studies have demonstrated that oral HMG does decrease

cholesterol synthesis and consequently results in lowered cholesterol concentrations in tissue and plasma (Beg and Lupien, 1972; Lupien et al., 1973; Van Niekerk et al., 1984).

Therefore, it was proposed that the increased plasma leucine associated with obesity or diabetes might increase cholesterol synthesis and cause the hypercholesterolemia commonly associated with these diseases (Paul and Adibi, 1980; Paxton and Harris, 1984). There are, however, no reports of dietary leucine altering cholesterol metabolism.

As discussed previously, dietary leucine is catabolized primarily in noncholesterol synthesizing tissues (peripheral tissues), whereas dietary KIC is catabolized in the gut and liver, which are most important in cholesterol synthesis. Thus, dietary leucine would be expected to have relatively little effect on cholesterol metabolism, whereas dietary KIC would be more likely to interact with cholesterol metabolism.

In an experiment designed to examine the effect of dietary KIC on rat cholesterol metabolism, feeding .1% and .5% KIC reduced aortic cholesterol concentration 64 and 71% (Flakoll and Nissen, 1986). One explanation given for this effect is that HMG produced from KIC inhibits HMG CoA reductase activity and cholesterol synthesis. However, KIC did not lower plasma cholesterol, whereas HMG does. Another mechanism postulated was that KIC may alter the metabolism of low-density lipoproteins and high-density lipoproteins, thereby changing cholesterol transport and deposition without changing total

plasma cholesterol content. Work demonstrating that synthesis of the liver-derived plasma protein albumin was increased with addition of KIC to liver perfusate (Kirsch et al., 1976) suggests KIC could alter lipoprotein metabolism. Finally, KIC can also be converted in the cytosol to β-hydroxy-β-methylbutyrate via KIC oxygenase, which has been found to inhibit HMG CoA reductase in vitro (Fimognari and Rodwell, 1965).

Effect of KIC on Hormones

Hormones that are known to alter and control growth and metabolism have been demonstrated to be affected by KIC. Insulin secretion has been shown by several researchers to be increased by KIC (Grill, 1982; Hutton and Malaisse, 1977; Holze and Panten, 1979; Stumpf and Kraus, 1978; Biden and Taylor, 1983; Hutton et al., 1974; Panten et al., 1974; Jain et al., 1978; Zawalich et al., 1978; Leclercq-Meyer et al., 1979a, b; Zawalich et al., 1979; Leclercq-Meyer et al., 1981; Lebrun et al., 1982; Lenzen et al., 1982; Sener et al., 1982). Conversely, KIC has been demonstrated to inhibit glucagon secretion (Leclercq-Meyer et al., 1979a, b; Sener et al., 1982). Stewart et al. (1984) noted that BCAA infusion increased the peak frequency of growth hormone release in baboons, whereas Cross and Walker (1984) and Miller et al. (1986) reported leucine administration to stimulate prolactin synthesis and increase the frequency of its release.

Nutritional Replacement of Leucine by KIC

Replacing a dietary essential amino acid with its corresponding keto acid is not a new concept. The α-keto analogues of histidine (Harrow and Sherwin, 1926) and tryptophan were (Berg et al., 1929; Jackson, 1929) found to promote growth in rats fed diets deficient in tryptophan. Reports of replacement of other amino acids by ketoacid supplementation occurred later (Meister, 1951; Wretlind, 1953; Cahill and Rudolph, 1942; Bubl and Butts, 1949; Wood and Cooley, 1954)

Several studies with rats examining the efficiency at which KIC replaces leucine in the diet have indicated that KIC is about 20 to 25% effective at replacing leucine (Chawla and Rudman, 1974; Chow and Walser, 1974; Kang and Walser, 1985; Chawla et al., 1975).

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EXPLANATION OF DISSERTATION FORMAT

This dissertation is presented in the alternate format, as outlined in the Iowa State Graduate College Thesis Manual. Use of the alternate format allows for the preparation of independent sections that are suitable for submission to scientific journals.

Three separate papers have been prepared from the data collected from research performed to partly fulfill requirements for the Ph.D. degree. Each paper is complete in itself and has an abstract, introduction, materials and methods, results, discussion, and literature cited section. The closeness of the subject matter of the three papers allowed a general discussion to be prepared.

ANIMAL CARE

The animals used in the following trials were treated in accordance with the Iowa State University animal care guidelines.

Influence of parenteral and oral administration of α-ketoisocaproate on lamb growth, feed conversion and carcass composition[1]

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[1]Journal Paper No. J-12927 of the Iowa Agriculture and Home Economics Experiment Station, Ames; Project 2614. This work was supported in part by NIH Grant AM32540 and a grant from Grain Processing Corp., Muscatine, Iowa.

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[3]The authors gratefully acknowledge K. Walker, S. Benson, S. Findlay, J. Cain, S. Rogers and D. Williams for their able technical assistance and care of animals.

SECTION I. INFLUENCE OF PARENTERAL AND ORAL ADMINISTRATION OF α-KETOISOCAPROATE ON LAMB GROWTH, FEED CONVERSION AND CARCASS COMPOSITION

Abstract

Two experiments were conducted to determine if α-ketoisocaproate (KIC), which is thought to be a regulating factor of metabolism, would influence lamb growth, feed conversion and nutrient deposition. In the first experiment (Exp. 1), daily intraperitoneal injections of a Na-KIC solution (~.11 g NaKIC/(kg body weight·d)) increased lamb growth 11% ($p<.09$) above the saline-treated lambs. In the second experiment (Exp. 2), when 1% Ca $(KIC)_2$ was fed with a soybean meal-supplemented diet (SBM), growth was increased 10%, whereas when 1% Ca $(KIC)_2$ was fed with a corn gluten meal supplemented diet (CGM), growth was reduced 10% (diet X KIC probability of > F = .001). Daily feed intake was reduced 10% when KIC was fed with CGM but was not changed when fed with SBM (diet X KIC probability of > F = .04). Oral administration of KIC increased wool growth irrespective of the diet fed (19% increase, $p<.03$). Backfat thickness was lowered by 28% in Exp. 1 ($p<.06$) and by 18% in Exp. 2 ($p<.06$) with KIC treatment. Fat in the lean tissue was decreased by 16% ($p<.05$) in Exp. 1 with KIC treatment. KIC-treated lambs in Exp. 1 had increased bone density ($p<.01$) and bone mineral density ($P<.03$). In Exp. 1, KIC tended to decrease plasma phosphorus 5% ($p<.33$). In Exp. 2 KIC decreased plasma phosphorus 16% when fed with SBM, but did not alter plasma phosphorus when fed with CGM (diet X KIC probability of > F = :05). These data indicate that KIC influences lamb metabolism such that parenteral administration improves daily gain and feed conversion and

decreases subcutaneous and intramuscular fat depots, whereas oral administration improves daily gain and feed conversion and reduces subcutaneous fat when fed with conventional diets, but depresses feed intake, daily gain and feed conversion when fed with corn gluten meal, which is high in leucine content.

(Keywords: Sheep, α-Ketoisocaproate, Growth, Fat Deposition.)

INTRODUCTION

The α-keto acid of leucine, α-ketoisocaproate (KIC), has been implicated as a regulating factor in metabolism. KIC has been shown in vitro to reduce protein breakdown (Chua et al., 1979; Tischler et al., 1982) and stimulate protein synthesis (Kirsch et al., 1976). Short-term nitrogen balance studies in humans have demonstrated that KIC spares nitrogen under conditions such as injury, starvation or kidney malfunctions (Sapir and Walser, 1977; Ell et al., 1978; Sapir et al., 1983; Attman et al., 1984). In addition to the effects of KIC on protein metabolism, it has been reported to inhibit glucagon secretion (Leclercq-Meyer et al., 1979; Sener et al., 1982) and stimulate insulin secretion (Panten et al., 1974; Holze and Panten, 1979). Either of these effects occurring in vivo should favorably affect protein deposition.

Protein turnover requires a significant expenditure of energy (Millward et al., 1975). Therefore, a reduction of proteolysis by KIC also should have an energy-sparing effect. The reported sparing of glucose by KIC is an example of such energy sparing (Stumpf et al., 1978). By sparing protein or glucose, KIC administration to meat-producing animals would minimize catabolism of amino acids and allow more energy to be available for increased fat or lean tissue growth. Therefore, two experiments were conducted to answer the following question: When KIC is administered to growing lambs, which are thought to have a relatively high protein catabolism rate, will there be increased growth, improved feed conversion and altered nutrient deposition in the carcass?

MATERIALS AND METHODS

Experiment 1

In the first experiment (Exp. 1), medium-frame crossbred rams weighing 17 kg were blocked by weight and randomly allotted to either a 60% corn grain diet (CG, Table 1) or a 30% corn grain plus 30% corn gluten feed diet (CGF, Table 1) and intraperitoneal injections of either saline or Na-KIC in a 2 X 2 factorial arrangement. Diets were corn-soybean meal based, balanced with vitamin and trace mineral premixes and calculated to be equivalent with respect to metabolizable protein and energy. Nine lambs were fed the CG diet, and nine lambs were fed the CGF diet.

Daily intraperitoneal injections were made for approximately 120 days. After disinfection with iodine, injections were made in the right lower abdomen. Nine lambs were injected with 50 ml saline, and nine lambs were injected with 50 ml of a NaKIC solution. The concentration of NaKIC in the solution was increased in proportion to animal weight (~.11 g NaKIC/(kg body weight·d)) with an average of 3.5 g of KIC being injected daily (4 to 10% NaKIC solutions).

The lambs were housed in individual Fiberglas pens, which were indoors with light and temperature controlled. Free access to water was given with nipple waterers and feed was given twice daily in quantities ensuring that feed was available to the lambs at all times. The lambs were allowed to adjust to the pens and environment for 2 weeks, during which time they were fed the CG diet.

Gain and feed intake were recorded biweekly. Lamb weights were

Table 1. Composition of basal diets (% of total mix, as fed)

Ingredient	Exp. 1 CG[a]	Exp. 1 CGF[b]	Exp. 2 SBM[c]	Exp. 2 CGM[d]
Corn grain	60.00	30.00	48.20	48.20
Dehydrated alfalfa[e]	-	-	20.00	20.00
Expeller soybean meal[f]	30.00	28.50	25.00	-
Corn gluten meal	-	-	-	17.20
Dry corn gluten feed[g]	-	30.00	-	-
Molasses, sugarcane[h]	6.70	6.70	5.00	5.00
Corn oil	.40	.90	-	.50
Corn starch	-	1.00	-	7.30
Limestone	1.50	2.00	.88	.88
Dicalcium phosphate	.50	-	-	-
Salt	.50	.50	.50	.50
Vitamin A premix[i]	.10	.10	.10	.10
Trace mineral premix[j]	-	-	.02	.02
Ammonium chloride	.30	.30	.30	.30
Calculated:				
Net energy-maintenance, Mcal[k]	1.97	1.92	1.86	1.85
Net energy-gain, Mcal[k]	1.33	1.29	1.22	1.20
Metabolizable protein, %[l]	12.3	12.9	11.0	10.4
Leucine, %[k]	2.08	2.35	2.01	3.04

[a]Exp. 1 diet containing 60% corn grain.

[b]Exp. 1 diet containing corn gluten feed.

[c]Exp. 2 diet containing soybean meal.

[d]Exp. 2 diet containing corn gluten meal.

[e]20% crude protein.

[f]Soy-Plus, West Central Cooperative, Ralston, IA.

[g]Dry corn gluten feed, Cargill, Inc., Cedar Rapids, IA.

[h]Mazola, CPC International Inc., Englewood Cliffs, NJ.

[i]5,200,000 IU vitamin A/kg, Hoffmann-LaRoche, Inc., Nutley, NJ.

[j]Ca 13-15%; Zn 12%; Mn 8.0%; Fe 10%; Cu 1.5%; I .2%; Co .1%, Calcium Carbonate division of J. M. Huber Corporation, Quincy, IL.

[k]Calculated from National Research Council values (National Research Council, 1982).

[l]Calculated from Burroughs et al., 1974.

recorded after a 12- to 16-hr fast. Jugular blood samples were taken at 4-wk intervals. When a lamb reached 50 kg, it was killed and internal organs were dissected, examined, weighed and sampled. Longissimus area (LA) at the 12th rib was traced and measured with a planimeter. Fat depth at the 12th rib interface was measured. The carcasses were physically separated into fat, lean and bone tissues with each portion being weighed. The lean tissue was ground through a .95-cm die and sampled. To measure fat-free wool growth, the lambs were sheared at the start of the trial and after 10 wks of treatment by a professional shearer.

Statistical analyses were performed on the variables from Exp. 1 by analysis of variance of the 2 X 2 factorial model by using the general linear models procedure of SAS (1985). Comparisons between treatment groups were made by t-test.

Experiment 2

Experiment 2 (Exp. 2) consisted of two replicated trials. In the first trial, 15 ewes and wethers weighing 29 kg were fed diets supplemented with either corn gluten meal or soybean meal and either 0 or 1% of the diet as Ca $(KIC)_2$ in a 2 X 2 factorial arrangement. In the second trial, 21 rams weighing 21 kg were again fed diets supplemented with either corn gluten meal or soybean meal and either 0 or 1 % of the diet as Ca $(KIC)_2$.

The lambs were housed, fed and watered as in Exp. 1. The diets were isocaloric and equal in metabolizable protein (Table 1). The major

difference between the diets was the amino acid composition; the corn gluten meal diet was calculated to contain 3.04% leucine, whereas the soybean meal diet was calculated to contain 2.01% leucine.

Experimental protocol for both trials was the same. The lambs were weighed and feed intake was recorded every 2 wk. Blood was sampled every 3 wk. When a lamb weighed 50 kg, it was killed and internal organs were dissected, examined, weighed and sampled. Backfat depth, LA and carcass fat, lean and bone were measured as in Exp. 1. Wool growth was measured by clipping a known marked area on each side of each lamb both at the start and at the conclusion of the trials.

Statistical analyses were performed on the variables from the experiment by analysis of variance of the replicated 2 X 2 factorial model by using the general linear models procedure of SAS (1985) and examining the effect of KIC, diet and KIC X diet interaction. Because there were no significant interactions between the trials and treatments (that is, the variables measured consistently followed the same trends in both trials), the results will be reported as the means of both trials.

Analytical Methods

Materials Na-KIC and Ca $(KIC)_2$ were obtained from SOBAC (336 Rue Saint-Honore, 75001, Paris, France).

Soft tissue analysis Fat and moisture were determined on the meat samples by using Goldfish extraction and lyophilization, respectively, according to AOAC procedures (AOAC, 1975). Crude protein

Tecator Kjeltec System 1003 distilling unit.

Wool analysis After an initial weighing, wool was rinsed with water to remove dirt and debris, dried at 60° C and reweighed. The wool was then rinsed with chloroform to extract fat, dried at 60° C and reweighed to obtain a fat-free wool weight.

Bone Analysis The humerus was dissected from the lambs in Exp. 1, and a 5.08-cm section from the exact center of the bone was removed, stripped of connective tissue and marrow and was measured for volume via planimeter tracing. The sample was weighed, dried at 80° C and reweighed. Bone density was calculated as the dried sample weight divided by the sample volume. The bone sample was ashed in a muffle furnace at 600° C and weighed to obtain ash weight. Ash density was calculated as the sample ash weight divided by the sample volume.

Plasma metabolite analysis Plasma leucine and KIC were analyzed by adding 20 nmol α-ketocaproate (KC) and 150 nmol norleucine (NL) as internal standards to 1 ml plasma. After deproteinizing with .75 N perchlorate and separation of amino and keto acids via cation column (Nissen et al., 1982), the extracts were dried under nitrogen gas and derivatized with 100 ul pyridine plus 100 ul N-methyl-N (t-butyl-dimethylsilyl) trifluoroacetamide containing 1% t-butyldimethyl-chlorosilane (Regis Chemical Company, Morton Grove, IL). KIC and KC were monitored at 301 AMU, and leucine and NL were monitored at 302 AMU by using a gas chromatography-mass spectrometry system (Hewlett-Packard 5970A). Concentrations were calculated by comparing the KIC to KC ratios and leucine to NL ratios with that of the standards.

An automated system (Rotochem IIA, Travenol Laboratories, Deerfield, IL) was used with enzymatic and colorimetric kits for determination of plasma glucose (Glucose (HK) Reagent, Gilford Systems, Oberlin, OH), blood urea nitrogen (BUN; BUN Reagent, Gilford Systems, Oberlin, OH), albumin (SpecTru BCG Albumin Reagent, Pierce Chemical Company, Rockford, IL), calcium (Rotochem Calcium, Pierce Chemical Company, Rockford, IL), and phosphorus (Worthington Inorganic Phosphorus Reagent Set, Cooper Biomedical, Inc., Malvern, PA). Because plasma from Exp. 1 and Trial 2 in Exp. 2 was collected into tubes with EDTA, calcium values from these trials were not reported.

Plasma hormone analysis Plasma was analyzed for cortisol concentration by radioimmunoassay (Amerlex Cortisol Kit, Amersham Corporation, Arlington Heights, IL). Plasma growth hormone and insulin concentrations were determined by radioimmunoassay by using bovine growth hormone antibody and bovine insulin antibody, respectively (Trenkle, 1972).

RESULTS

The effect of KIC on lamb performance in Exp. 1 is reported in Table 2. When KIC was delivered intraperitoneally, lamb growth was improved 11% above the control ($p<.09$). Neither daily feed intake nor feed conversion were altered by KIC treatment. Intraperitoneal doses of KIC reduced wool growth by 14% ($p<.09$).

Lamb performance in Exp. 2 is reported in Table 3. When 1% KIC was fed with the CGM-supplemented diet, daily gain was reduced 12%, whereas, when 1% KIC was fed with the SBM-supplemented diet, daily gain was increased 10% (diet X KIC probability of > F = .001). Daily feed intake was reduced 10% when KIC was fed with CGM, but was not changed when fed with SBM (diet X KIC probability of > F = .04). KIC depressed feed conversion by 4% when fed with CGM, whereas it improved feed conversion by 9% when fed with SBM (diet X KIC probability of > F = .02). Oral administration of KIC increased wool growth irrespective of the diet fed (19% increase, $p<.03$).

Lamb organ weight in Exp. 1 is reported in Table 4. When expressed as percentage of body weight, no change in carcass, liver, spleen, lungs or adrenal glands were noted. The weight of the heart and kidneys were increased 12% ($p<.01$) and 11% ($p<.07$), respectively. Lamb organ weight in Exp. 2 is reported in Table 5. When expressed as percentage of body weight, no change in carcass, liver, spleen, heart, lungs, adrenal glands or kidneys were noted.

The effects of intraperitoneal administration of KIC on lamb carcass composition are reported in Table 6. Perirenal fat and LA were

Table 2. Effects of daily intraperitoneal injections of α-ketoisocaproate (KIC) for approximately 120 days on lamb growth, feed conversion and wool growth (Exp. 1)

Parameter	Saline	KIC	SEM[a]	P<[b]
n	9	9		
Daily gain, g	293	324	12	.09
Daily intake, g	1330	1400	32	.15
Feed/gain	4.60	4.36	.17	.66
Fat-free wool gain mg/(cm^2·d)	.81	.70	.05	.08

[a]Pooled standard error of the mean.

[b]Probability that treatment means are not different.

Table 3. Effects of oral administration of α-ketoisocaproate (KIC) on growth, feed conversion and wool growth of lambs fed diets supplemented with corn gluten meal (CGM) or soybean meal (SBM; Exp. 2, Trials 1 and 2 combined)

	---Supplement (Suppl)----							
	---CGM----		----SBM---			------P > F-------		
	-----% Dietary KIC------							
Parameter	0	1	0	1	SEM[a]	Suppl	KIC	Suppl x KIC
n	9	9	10	8				
Daily gain, g	349	306	315	346	10	.71	.59	.001
Daily intake, g	1570	1430	1530	1520	32	.24	.04	.04
Feed/gain	4.50	4.68	4.87	4.41	.13	.58	.31	.02
Fat-free wool gain, mg/(cm^2·d)	.96	1.06	.87	1.11	.07	.62	.03	.42

[a]Pooled standard error of the mean.

Table 4. Effects of daily intraperitoneal injections of α-ketoisocaproate (KIC) for approximately 120 days on lamb organ weight (Exp. 1)

Parameter	Saline	KIC	SEM[a]	P<[b]
Final weight, kg	48.8	48.6	.2	.64
Carcass, %[c]	52.0	52.5	.8	.60
Liver, %[c]	2.0	2.2	.1	.13
Spleen, %[c]	.25	.25	.02	.93
Heart, %[c]	.40	.45	.01	.02
Lungs, %[c]	1.10	1.07	.07	.80
Kidneys, %[c]	.31	.34	.01	.07
Adrenal glands, %[c] X 1000	7.0	6.8	.1	.65

[a]Pooled standard error of the mean.

[b]Probability that treatment means are not different.

[c]Percentage of final body weight.

Table 5. Effects of oral administration of α-ketoisocaproate (KIC) on organ weight of lambs fed diets supplemented with corn gluten corn gluten meal (CGM) or soybean meal (SBM; Exp. 2, Trials 1 and 2 combined)

	---Supplement (Suppl)---							
	---CGM----		----SBM---			-------P > F------		
	-----% Dietary KIC------							
Parameter	0	1	0	1	SEM[a]	Suppl	KIC	Suppl x KIC
Final weight, kg	49.7	48.6	48.3	50.2	.7	.72	.53	.03
Carcass, %[b]	55.5	55.2	55.3	55.2	.8	.80	.79	.95
Liver, %[b]	2.1	1.9	1.9	1.9	.1	.22	.22	.28
Spleen, %[b]	.17	.21	.17	.17	.01	.15	.22	.24
Heart, %[b]	.39	.39	.40	.41	.01	.35	.73	.80
Lungs, %[b]	.83	.92	.96	.91	.05	.30	.65	.16
Kidneys, %[b]	.31	.29	.29	.31	.01	.65	.97	.14
Adrenal glands, %[b] X 1000	5.4	4.7	5.6	5.2	.3	.53	.26	.75

[a]Pooled standard error of the mean.

[b]Percentage of final body weight.

Table 6. Effects of daily intraperitoneal injections of α-ketoisocaproate (KIC) for approximately 120 days on lamb carcass composition (Exp. 1)

Parameter	Saline	KIC	SEM[a]	P<[b]
Longissimus area, cm^2	15.2	14.8	.8	.78
Backfat, mm	5.3	3.8	.5	.06
Perirenal fat, %[c]	1.5	1.3	.2	.52
Carcass:				
Separable lean, %[d]	55.2	53.0	.9	.12
Separable fat, %[d]	18.9	17.9	1.2	.55
Separable bone, %[d]	24.4	25.7	.9	.67
Lean:				
Dry matter, %[e]	30.9	29.0	.8	.10
Crude protein, %[e]	16.8	17.1	.5	.63
Ether extract, %[e]	14.0	11.7	.8	.05
Wool fat, %[f]	5.5	4.1	.9	.30
Bone (humerus):				
Dry matter, %[g]	86.8	86.5	.3	.53
Ash, %[g]	59.4	58.9	.3	.20
Density[h], g/cm^3	1.35	1.47	.03	.01
Ash density[i], g/cm^3	.92	1.00	.02	.03

[a]Pooled standard error of the mean.
[b]Probability that treatment means are not different.
[c]Percentage of final body weight.
[d]Percentage of carcass weight.
[e]Percentage of lean tissue weight.
[f]Percentage of wool weight.
[g]Percentage of bone weight.
[h]Dried humerus sample weight divided by the sample volume.
[i]Ashed humerus sample weight divided by the sample volume.

not altered by KIC treatment. Backfat thickness was lowered with KIC treatment by 28% ($p<.06$). The portions of the carcass separated into lean, fat or bone were not significantly altered. Fat in the lean tissue was decreased by 16% with KIC ($p<.05$). Wool fat was not significantly altered. Bone tissue dry-matter and ash content were not altered by KIC treatment, but KIC increased bone density 9% ($p<.01$) and bone mineral density 9% ($p<.03$).

The effects of oral administration of KIC on lamb carcass composition are reported in Table 7. KIC treatment did not alter LA. Perirenal fat was not altered by KIC treatment, but there was more perirenal fat for animals fed the SBM diet when compared with the CGM diet ($p<.008$). Backfat thickness was lowered by KIC for both diets (18% decrease, $p<.06$). The portions of the carcass separated into fat, lean or bone were not significantly altered by treatment. Fat in lean tissue and wool was not significantly altered by KIC.

Plasma parameters from lambs in Exp. 1 are reported in Table 8. Plasma KIC in the KIC-treated lambs was 31% lower than in the control lambs ($p<.03$). No significant changes in plasma leucine, glucose, BUN, albumin or phosphorus concentrations as a result of KIC treatment were noted. In addition, plasma concentrations of cortisol, growth hormone or insulin were not altered.

Plasma parameters from lambs in Exp. 2 are reported in Table 9. Plasma phosphorus was decreased 16% when lambs were fed KIC with the SBM diet, whereas KIC did not affect plasma phosphorus when fed with the CGM diet (diet X KIC probability of $> F = .05$). No significant changes in

Table 7. Effects of oral administration of α-ketoisocaproate (KIC) on carcass composition of lambs fed diets supplemented with corn gluten meal (CGM) or soybean meal (SBM; Exp. 2, Trials 1 and 2 combined)

	---Supplement (Suppl)---							
	---CGM----		----SBM---			-------P > F-----		
	-----% Dietary KIC------							
Parameter	0	1	0	1	SEM[a]	Suppl	KIC	Suppl x KIC
Longissimus area, cm^2	14.9	15.5	15.4	14.6	.6	.78	.94	.23
Back fat, mm	6.3	4.9	6.1	5.4	.5	.82	.06	.49
Perirenal fat, %[b]	1.80	1.34	2.11	2.03	.21	.008	.24	.33
Carcass:								
Separable lean, %[c]	50.7	51.0	51.1	50.8	1.2	.94	.98	.83
Separable fat, %[c]	25.8	26.3	27.2	25.9	1.3	.65	.78	.51
Separable bone, %[c]	23.5	22.7	21.7	23.3	.9	.43	.64	.19
Lean:								
Dry matter, %[d]	31.1	30.7	30.8	30.0	.7	.54	.44	.77
Crude protein, %[d]	18.5	19.1	18.7	18.0	.32	.15	.77	.06
Ether extract, %[d]	15.4	16.3	17.0	16.7	.75	.32	.77	.37
Wool fat, %[e]	21.5	19.8	21.0	20.6	1.5	.82	.51	.65

[a]Pooled standard error of the mean.

[b]Percentage of final body weight.

[c]Percentage of carcass weight.

[d]Percentage of lean tissue weight.

[e]Percentage of wool weight.

Table 8. Effects of daily intraperitoneal injection of α-ketoisocaproate (KIC) for approximately 120 days on lamb plasma parameters (Exp. 1)[a]

Parameter	Saline	KIC	SEM[b]	P<[c]
KIC, uM	25.4	17.4	2.4	.03
Leucine, uM	249	242	15	.74
Glucose, mg/dl	76.9	74.9	2.5	.58
BUN, mg/dl	31.1	30.0	1.2	.53
Albumin, mg/dl	4.00	4.06	.08	.55
Phosphorus, mg/dl	10.1	9.6	.3	.33
Cortisol, ug/dl	1.26	1.34	.20	.78
Growth hormone, ng/ml	2.83	3.49	.59	.55
Insulin, ng/ml	0.76	0.68	.17	.71

[a]Blood was sampled after an overnight fast and pooled for analysis.

[b]Pooled standard error of the mean.

[c]Probability that treatment means are not different.

Table 9. Effects of oral administration of α-ketoisocaproate (KIC) on blood parameters of lambs fed diets supplemented with corn gluten meal (CGM) or soybean meal (SBM; Exp. 2, Trials 1 and 2 combined)[a]

	---Supplement (Suppl)---							
	---CGM----		----SBM---			-------P > F-----		
	-----% Dietary KIC------							
Parameter	0	1	0	1	SEM[b]	Suppl	KIC	Suppl x KIC
KIC, uM	22.2	19.6	18.7	26.2	3.8	.65	.51	.19
Leucine, uM	347	317	237	231	20	.0001	.37	.60
Glucose, mg/dl	79.1	77.6	81.0	77.6	3.2	.77	.54	.77
BUN, mg/dl	28.6	25.3	26.7	24.4	2.1	.52	.20	.81
Albumin, mg/dl	3.81	3.87	3.96	3.58	.16	.66	.66	.18
Calcium, mg/dl[c]	10.7	10.8	10.9	11.1	.2	.30	.55	.96
Phosphorus, mg/dl	7.9	7.9	8.5	7.1	.3	.78	.06	.05
Cortisol, ug/dl	2.91	1.76	2.35	2.79	.47	.67	.46	.11
Growth hormone, ng/ml	1.01	1.69	1.26	1.32	.34	.85	.29	.37
Insulin, ng/ml	2.00	1.02	0.87	0.88	.24	.02	.06	.05

[a]Blood was sampled after 4 and 6 weeks of treatment after an overnight fast.

[b]Pooled standard error of the mean.

[c]Values are from Trial 1 only.

plasma KIC, leucine, glucose, BUN, albumin or calcium concentrations with KIC treatment were noted. However, plasma leucine concentration was increased 29% for lambs fed the CGM-supplemented diet ($p<.0001$). Plasma concentrations of cortisol or growth hormone were not altered. Although KIC had no effect on plasma insulin concentration in lambs fed the SBM diet, insulin was decreased 49% when KIC was fed with the CGM diet (diet X KIC interaction probability of $> F = .05$). Insulin concentration in the lambs fed the CGM diets were 42% higher than in the lambs fed the SBM diets ($p<.02$).

Discussion

KIC improved lamb gain and feed conversion in all experiments when fed with diets in which soybean meal was the supplemental source of protein. Overall gain was increased slightly more than 10%, whereas feed conversion was improved approximately 8%. Additionally, KIC reduced disposition of energy to internal and external fat depots. Backfat, renal fat, dissectible carcass fat and fat in the carcass lean tissue were all lower on KIC treatments. Together, these data suggest that KIC improves growth by decreasing fat synthesis and increasing protein synthesis.

The most likely mechanism for the effects of KIC points to its interaction with protein turnover. It has been estimated that 4 ATP of energy are required per peptide bond synthesized. Because protein turnover is an energy-requiring process, the energy expenditure of an animal can be significantly influenced by changes in protein metabolism

(Reeds et al., 1985). This is particularly true in ruminants, in which a larger protein mass has to be maintained and considerably more of the body weight consists of digestive tissue, which, when compared with other tissues, has a comparatively high rate of protein turnover. The energy cost of protein synthesis has been shown to be greater in ruminating than in nonruminating lambs (Rattray and Jagusch, 1977). Although Waterlow et al. (1978) estimated that protein synthesis made up 10 to 20% of total heat production in mature nonruminants, Davis et al. (1981) estimated that the energy cost of protein synthesis accounted for 42% of daily heat production in growing lambs. The rate of protein synthesis is approximately 20 fold that of protein deposition (Waterlow et al., 1978). Therefore, a small decrease in proteolysis because of KIC could result in a significant gain in protein deposition.

Additionally, with decreased proteolysis, fewer free amino acids would be available to be catabolized for energy use and other sources of energy (lipid and glucose) would have to be utilized. But previous studies have shown that glucose is also spared by KIC (Tischler and Goldberg, 1980). Therefore, more fats may need to be utilized as energy sources, and less would be stored.

Whether the alterations in fat deposition is a result of a direct or indirect effect of KIC on adipose tissue metabolism is unclear. In a study in which KIC was fed to lactating dairy cows, increased milk fat production was attributed to an increase in de novo fatty acid synthesis by the mammary gland and an increase in transport of blood lipids to the mammary gland (VandeHaar et al., 1988).

Another possible mechanism could be through altered hormone or hormone receptor activity. No differences in cortisol or growth hormone concentrations were noted in the experiments reported here, but blood was sampled only at one point in time and these hormones have been shown to be secreted episodically and at different rates as time from feeding, stress or other environmental circumstances change.

Insulin was not altered by KIC in Exp. 1, nor when fed with the SBM diet in Exp. 2. However, when KIC was fed with the CGM diet in Exp. 2, insulin levels were decreased. Therefore, when KIC increased growth rates of the lambs, insulin levels were not altered, but when KIC decreased growth, insulin levels were decreased. While KIC has been demonstrated to stimulate insulin secretion (Panten et al., 1974; Holze and Panten, 1979; Leclercq-Meyer et al., 1979; Sener et al., 1982), there are other reports in which a KIC load did not alter insulin concentration in dogs (Abumrad et al., 1982b) or humans (Sapir et al., 1983). Previous research has demonstrated leucine administration raises plasma insulin levels (Abumrad et al., 1982a), which is consistent with the results of feeding the CGM diet in Exp. 2. But the mechanism whereby KIC would decrease insulin levels under these conditions is unclear.

It could be argued that the growth response to KIC may be through a ruminal effect on microorganisms as has been shown with other short-chain fatty acids. Although in vitro results indicate that rumen microorganisms have requirements for branched-chain acids and that branched-chain acid supplementation increases rumen microorganism growth

(Socransky et al., 1964; Bryant, 1974; Allison, 1978), minimal or no increase in growth was observed in studies in which branched-chain acids were fed to ruminants (Hungate and Dyer, 1956; McCollum et al., 1986). Additionally, as perenteral KIC gave responses similar to oral KIC, KIC promoting growth through rumen microorganisms seems unlikely. However, a direct effect of KIC on intestinal and hepatic tissue metabolism cannot be discounted.

Whereas the structure and metabolic fate of KIC and leucine are similar, suggesting that KIC and leucine may produce similar physiological responses, it is unlikely that KIC acts through leucine. Although KIC has been implicated in altering nitrogen metabolism, its amino acid leucine has not exhibited many of these properties. The reduction of proteolysis by KIC is not mimicked by leucine when transamination is prevented (Chua et al., 1979; Tischler et al., 1982, Poso et al., 1982). When KIC infusions were given to postoperative patients on no other source of calories, proteolysis was suppressed, and the postoperative fall in plasma levels of pre-albumin and retinol-binding protein seen in controls was significantly reduced (Sapir et al., 1983). No response was observed with leucine infusions. Herlong et al. (1980) observed significant improvement in grade of encephalopathy, electroencephalographic abnormalities and hyperammonaemia when ornithine salts of branched-chain keto acids were administered orally for 5 days to encephalopathic patients. Significantly lesser effects were observed in similar patients treated with branched-chain amino acids. Nitrogen was significantly spared in fasted obese men infused with KIC when compared

with controls or leucine-infused treatments (Mitch et al., 1981).

Although it is unlikely that KIC acts through leucine, it is unclear whether KIC acts through any of its breakdown products. Both ketone bodies (Sherwin et al., 1975) and isovalerate (Chua et al., 1979) have been shown to decrease proteolysis.

Oral doses of KIC increased wool growth, irrespective of the diet fed, whereas KIC decreased wool growth when delivered intraperitoneally, irrespective of the diet fed. This may be a reflection of the site of KIC metabolism. An oral dose would undergo extensive ruminal degradation, considerable splanchnic uptake, with a small amount of KIC reaching the periphery. It has been demonstrated in dogs that 85% of an oral dose of KIC is metabolized by the splanchnic tissues (Abumrad et al., 1982b; Bassler and Pietrek, 1983). Much more of an intraperitoneal load of KIC would reach the peripheral tissues. Peripheral uptake of KIC has been shown to be rapid and extensive (Pozefsky and Walser, 1977; Bassler and Pietrek, 1983). In the present experiments, daily doses of 3 g of KIC intraperitoneally and 15 g of KIC orally with a diet not excessive in leucine produced equivalent responses in gain.

Bone in the carcass seemed to increase when KIC was injected or fed with the SBM-supplemented diet. In addition, bone density (Exp. 1) was increased by KIC. In Exp. 2, blood calcium was not changed, but blood phosphorus was decreased. Therefore, the blood calcium to phosphorus ratio was increased. When KIC and other keto acids were administered to patients with chronic renal failure or uremia, it was noted that plasma phosphate concentrations were decreased (Walser, 1975;

Heidland et al., 1978; Frohling et al., 1980; Hecking et al., 1980; Kampf et al., 1980; Attman et al., 1984). One explanation given was that this decrease was the result of the anabolic effect of the ketoacid treatment. Heidland et al. (1978) postulated that the reduced serum phosphate was the result of decreased phosphate intake or increased fecal phosphate excretion because of formation of calcium-phosphate complexes in the gut. This alteration of phosphorus metabolism may involve the reduction in parathyroid concentrations. There several previous reports of parathyroid concentration decreasing during ketoacid therapy (DiLandro et al., 1982; Schmicker et al., 1982; Lindenau et al., 1982; Barsotti et al., 1982). Although most clinical studies reported no change in blood calcium, there was one report of hypercalcemia due to keto acid infusion (Attman et al., 1984). The reason for this observation was attributed to increased calcium intake with Ca $(KIC)_2$ treatment. There were not any differences in blood calcium reported when Ca $(KIC)_2$ was fed in Exp. 2, but the amount of calcium fed was maintained constant between treatments.

Although leucine and KIC are catabolized through the identical biochemical pathway, the organs by which they are metabolized are distinctly different. The liver is limiting in branched-chain amino acid transaminase, so very little dietary leucine is catabolized until it reaches extrahepatic tissues (Ichihara and Koyama, 1966; Krebs and Lund, 1977). Conversely, the liver contains abundant quantities of branched-chain ketoacid dehydrogenase (Shinnick and Harper, 1976), the enzyme responsible for the first irreversible step of KIC oxidation.

Lambs that received the CGM-supplemented diet had significantly higher blood concentrations of leucine than did the lambs receiving the SBM-supplemented diet. The ruminant species may be more responsive to dietary leucine in terms of blood leucine concentration because of reduced transamination when compared with other species of animals (Nissen and Ostaszewski, 1985; Pell et al., 1986). Another difference between lambs on the SBM and CGM diets were the lowered amount of renal fat for lambs fed the CGM diet. The reasons for this are unclear.

When KIC was fed with SBM, feed intake was not affected. However, when KIC was fed with CGM, feed intake was significantly reduced by 10%, suggesting a negative interaction of KIC with high leucine dietary intake. The lowered intake was reflected in lamb gain, which was reduced 12% when KIC was fed with CGM. Conversely, when KIC was fed with SBM, gain was significantly increased 12%. In addition, when fed with CGM, KIC worsened feed conversion by 6%, but when fed with SBM, KIC improved feed conversion by 9%.

In conclusion, the increased growth and decreased fat deposition in lambs administered KIC suggests that KIC may alter energy partition between fat and lean tissue, possibly through its control of protein metabolism. Feeding KIC improves performance and alters carcass composition of lambs similarly to injecting KIC when KIC is fed with diets not excessive in leucine (conventional lamb diets). These data demonstrate that increased rate of growth, reduced disposition of energy to internal and external fat depots and increased bone density are achieved by administering KIC to growing lambs.

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Influence of feeding α-ketoisocaproate protected from ruminal degradation on growth, feed conversion and carcass composition in steers and lambs[1]

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[1]Project 2614. This work was supported in part by NIH Grant AM32540 and the Iowa Agriculture and Home Economics Experiment Station, Ames.

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[3]Appreciation is expressed to S. Feurbach, J. Hahn, D. Cain, R. Berryman and the farm crew at the ruminant nutrition research farm for animal care, J. Cain, S. Rogers, T. Kuan, C. Telleen, B. Beer and D. Williams for their technical assistance, T. Elsasser at the USDA-ARS, Beltsville, MD, for measuring IGF-I concentrations and Caldwell Packing Co., Windom, MN. for their cooperation.

SECTION II. INFLUENCE OF FEEDING α-KETOISOCAPROATE PROTECTED FROM RUMINAL DEGRADATION ON GROWTH, FEED CONVERSION AND CARCASS COMPOSITION IN STEERS AND LAMBS

Abstract

Two experiments were conducted to examine if feeding α-ketoisocaproate (KIC) protected from ruminal degradation would alter gain, feed conversion or carcass composition of cattle and sheep. In Experiment 1 (Exp. 1), 72 steers in 12 pens were fed a basal diet plus either 0, .02, .07 or .2% $Ca(KIC)_2$. Experiment 2 (Exp. 2) consisted of two replicated trials in which lambs were fed .05% leucine (n=12), KIC (n=16), isovalerate (IVA; n=12) or limestone (control; n=13). In both experiments, treatment supplements were combined with zein, and pellets were formed that were resistant to ruminal degradation. Over the 161-d Exp. 1, cattle growth was increased in quadratic dose-responsive manner ($p<.03$) with .02% KIC increasing gain by 14%. The number of cattle grading choice or prime was increased linearly by KIC ($p<.02$). Cattle plasma glucose, glucagon and cortisol were linearly decreased by KIC ($p<.02$, $p<.09$ and $p<.02$, respectively), whereas insulin/glucagon was linearly increased ($p<.06$). Compared with controls, KIC decreased lamb backfat 17% ($p<.04$) and increased weights of psoas 11% ($p<.07$), longissimus 8% ($p<.05$), gastrocnemius 12% ($p<.07$), tibia 5% ($p<.09$) and humerus 12% ($p<.006$). Leucine increased weights of tibia 5% ($p<.08$) and humerus 10% ($p<.06$). Supplemental KIC, protected from ruminal degradation, has potential to improve cattle and lamb growth and alter body composition at doses 10-to-20 fold lower than when fed unprotected.

Keywords: Sheep, Cattle, α-Ketoisocaproate, Growth, Carcass composition.

INTRODUCTION

Previous research has demonstrated that α-ketisocaproate (KIC) administered to lambs intraperitoneally at 3 g daily or orally at 15 g daily improved improved gain and feed conversion approximately 10% (Flakoll and Nissen, 1986; Flakoll et al., 1986). Additionally, internal fat and backfat depots were reduced by KIC treatment in these lambs. KIC also has been shown to increase milk fat production in dairy cattle (VandeHaar et al., 1988). In these trials, intraperitoneal doses of KIC were found to have equivalent growth-promoting effects at a dose one-fifth that of oral trials. Similar to free amino acids, KIC is readily degraded in the rumen. Therefore, if KIC-ruminal loss is reduced, the dose required to produce positive growth effects would be reduced.

Several methods have been proposed to protect compounds from ruminal degradation. These include lipid encapsulation (Smith and Boling, 1984), coating with blood meal (Mir et al., 1984), coating with fish hydrolysate (Mir et al., 1984) and encapsulation with a styrene and 2-methyl-5-pyridine copolymer (Dannelly and Ardell, 1980a, b; Papas et al., 1984). However, these methods are not optimal in that they do not result in optimal by-pass, nor are they available for small-scale production. Therefore, a new technique was developed, whereby the rumen-stable protein zein, was combined with KIC and other metabolites and formed into pellets. Approximately 60% of KIC and methionine were estimated to bypass the rumen with this technique (VandeHaar et al., 1987).

Therefore, the objective of this research was to answer the following questions: 1) does oral administration of KIC, protected from ruminal degradation, alter steer and lamb growth rates and carcass composition as effectively as intraperitoneal or unprotected oral administration; 2) will the optimal dose for these effects be lower than for intraperitoneal or unprotected oral administration; 3) do leucine, KIC and isovalerate (IVA) invoke similar responses; and 4) is the effect of KIC mediated by changes in growth regulating hormones, such as insulin and glucagon for which secretion is known to be altered by KIC (Leclercq-Meyer et al., 1979; Panten et al., 1974; Holze and Panten, 1979; and Sener et al., 1982)?

MATERIALS AND METHODS

Experiment 1

In the first experiment (Exp. 1), 72 medium-frame cross-bred steers, with an average weight of 263 kg, were blocked by weight and allotted to 12 pens of 6 animals each. Three pens were assigned to each treatment. A basal diet consisting of mainly corn and soybean meal was fed ad libitum to all cattle (Table 1). The diet was high in energy and protein to maintain a high level of growth. The treatments consisted of four levels of Ca $(KIC)_2$ in a pelleted supplement (Table 2): 0%, .02%, .07% and .20% of the basal diet as KIC (.05% control pellets, .05% KIC pellets, .175% KIC pellets and .50% KIC pellets). The pellets were preweighed and top-dressed on the basal diet twice daily. After 72 d, the cattle on the .20% KIC treatment were removed from the experiment.

Neither implanted growth promotants nor ionophores were included in this study. The cattle were placed in the feedlot and fed a growing ration for 5 wk prior to the start of the experiment. Weights were recorded on 2 consecutive d at the beginning and end of the experiment, and every 14 d during the trial. Jugular blood samples were collected into sodium EDTA-coated tubes and centrifuged. Plasma was stored at -70° C.

The cattle were sold as one complete group to a commercial beef processing plant when they were appraised by visual examination to grade Choice. Longissimus area and fat thickness over the longissimus at the 13th rib were measured on each carcass. Quality and yield grades and the percentage of perirenal, pericardial and pelvic fat were estimated

Table 1. Composition of basal diets for steers in Exp. 1 (% of total mix, as fed)

Ingredient	0-72 Days (~263-378 kg)	72-161 Days (~378-485 kg)
Corn grain	48.9	54.3
Corn cobs	24.4	24.4
Expeller soybean meal[a]	17.8	12.8
Molasses, sugarcane	7.0	7.0
Limestone	1.3	1.3
Dicalcium phosphate	.25	.25
Salt	.2	.2
Trace mineral premix[b]	.02	.02
Vitamin A premix[c]	.1	.1
Calculated:		
Dry matter, %[d]	88	88
Crude protein, %[d]	15	13
Metabolizable protein, g/kg[e]	86	76
Leucine, %[d]	1.7	1.7
Net energy- maintenance, Mcal/kg[d]	1.7	1.7
gain, Mcal/kg[d]	1.1	1.1

[a]Soy-Plus, West Central Cooperative, Ralston, IA.
[b]Ca, 13-15%; Zn 12%; Mn 8.0%; Fe 10%; Cu 1.5%; I .2%; Co .1%; Calcium Carbonate division of J. M. Huber Corporation, Quincy, IL.
[c]5,200,000 IU Vitamin A/kg, Hoffmann-LaRoche, Inc., Nutley, NJ.
[d]Calculated from National Research Council values (1982).
[e]Calculated from Burroughs et al. (1974).

Table 2. Composition of treatment supplements in Exp. 1 and 2[a]

Ingredient	Leucine	KIC	IVA	Control
	----------g/kg----------			
Zein[b]	450	450	450	450
Ethanol	278	278	278	278
Water	37	37	37	37
Limestone,[c]	16	10	8	45
Sodium Bentonite,[d]	5	5	5	10
Leucine[e]	340			
α-Ketoisocaproate[f]		400		
Isovalerate[g]			420	

[a]Dried treatment pellets contained approximately 2.5 mole leucine, KIC or IVA, and 64, 91, 97 or 180 g calcium per kg leucine, KIC, IVA or control pellets, respectively.

[b]Freeman Industries, Tuckahoe, NY.

[c]325 mesh; Iowa Limestone Company, Des Moines, IA.

[d]200 mesh; American Colloid Company, West Des Moines, IA.

[e]Sigma Chemical Company, St. Louis, MO.

[f]Ca (α-ketoisocaproate)$_2$ H_2O; SOBAC, Rue Saint-Honore, 75001, Paris, France.

[g]Ca (isovalerate)$_2$ H_2O prepared from isovaleric acid (Sigma Chemical Company, St. Louis, MO) as described in Materials and Methods.

by federal graders.

Experiment 2

The second experiment (Exp. 2) consisted of two replicate trials. In the first trial, 30 mixed-breed ram lambs, weighing 21 kg, were randomly allocated to one of four dietary treatment groups: 1) leucine (n=7), 2) KIC (n=8), 3) IVA (n=8) or 4) limestone (control; n=8). In the second trial, 30 mixed-breed ram lambs, weighing 24 kg, were randomly allotted to one of four dietary treatments 1) leucine (n=7), 2) KIC (n=8), 3) IVA (n=7) and 4) limestone (control; n=8). All animals were fed a corn-soybean meal basal diet (Table 3) supplemented with 5 g ammonium chloride daily. The lambs were caged individually in a light- and temperature-controlled environment with free access to water.

Dietary treatments consisted of leucine, KIC, IVA or limestone (control), which were supplemented to the basal diet twice daily as zein-coated pellets (Table 2). Lambs were fed their respective pellets at a rate of 1 g/d for the first 6 wk, 1.5 g/d for wk 6-10 and 2 g/d for wk 10 to time of slaughter. These doses translate to about 1 g pellets per kg diet and 2.5 mmol leucine, KIC or IVA per kg diet (molar equivalent of .05% leucine added to the diet).

Lambs were weighed weekly and total feed consumption for each 2 wk period was recorded. Jugular blood samples were collected after 4 and 5 wk of treatment into polypropylene tubes, allowed to coagulate and centrifuged. Serum was stored at -70^{o} C.

Upon reaching approximately 50 kg body weight, lambs were fasted 18

Table 3. Composition of basal diet for lambs in Exp. 2 (Trials 1 and 2)

Ingredient	% of total mix (as fed basis)
Corn grain	60
Expeller soybean meal[a]	21
Dehydrated alfalfa meal[b]	10
Molasses, sugarcane	7.5
Corn oil[c]	.38
Limestone	.50
Salt	.50
Vitamin A premix[d]	.10
Trace mineral premix[e]	.02
Calculated:	
Dry matter, %	88
Crude protein, %[f]	17
Metabolizable protein, g/kg[g]	124
Leucine, %[f]	1.8
Net energy- maintenance, Mcal/kg[f]	1.9
gain, Mcal/kg[f]	1.3

[a]Soy-Plus, West Central Cooperative, Ralston, IA.
[b]20% crude protein.
[c]Mazola, CPC International., Englewood Cliffs, NJ.
[d]Ca, 13-15%; Zn 12%; Mn 8.0%; Fe 10%; Cu 1.5%; I .2%; Co .1%; Calcium Carbonate division of J. M. Huber Corporation, Quincy, IL.
[e]5,200,000 IU Vitamin A/kg, Hoffmann-LaRoche, Nutley, NJ.
[f]Calculated from National Research Council values (1982).
[g]Calculated from Burroughs et al. (1974).

hr and killed by electrical shock and exsanguination. Wool growth was measured by clipping a known marked area on both sides of the lamb both at the start and at the conclusion of the trials. Carcass, perirenal fat, left and right gastrocnemius muscles, left and right psoas major muscles and left and right longissimus dorsi muscles (posterior to 10th rib) were separated and weighed. Carcasses were severed between the 10th and 11th ribs; backfat thickness and longissimus area were measured. The right tibia and right femur were separated and cleaned, and length and weight were recorded.

Treatment Supplement Preparation

Treatment pellets, resistant to ruminal degradation, were made by thoroughly mixing zein and 88% ethanol and adding limestone, bentonite and either Ca $(KIC)_2$, Ca $(IVA)_2$, leucine or more limestone in the ratios presented in Table 2. This mixture was extruded through a 3-mm die directly into a vat of liquid nitrogen for freeze-fracturing. The frozen, shattered pellets were allowed to dry slowly at room temperature. After drying, the pellets were shaken under forced air and sprayed with a solution of 20% zein in 88% ethanol (100 ml/kg pellets). Average pellet size was 2 x 8 mm, and density was 1.1 to 1.2 g/ml. Ruminal incubation of the pellets in a dacron bag indicated that ruminal bypass of the zein-protected compounds was approximately 60% (VandeHaar et al., 1987). Ca $(IVA)_2$ was made by combining slightly excess calcium hydroxide on a molar basis with IVA. The salt produced was calculated to be 73% Ca $(IVA)_2$.

Wool Analysis

After an initial weighing, wool was rinsed with water to remove dirt and debris, dried at 60° C and reweighed. The wool was then rinsed with chloroform to extract fat, dried at 60° C and reweighed to obtain a fat-free wool weight.

Plasma Metabolite Analysis

Plasma leucine and KIC were analyzed by adding 20 nmol α-ketocaproate (KC) and 150 nmol norleucine (NL) as internal standard to 1 ml plasma. After deproteinizing with .75 N perchlorate and separation of amino and keto acids via cation exchange column (Nissen et al., 1982), the extracts were dried under nitrogen gas and derivatized with 100 ul pyridine plus 100 ul N-methyl-N (t-butyl-dimethylsilyl) trifluoroacetamide containing 1% t-butyldimethyl-chlorosilane (Regis Chemical Company, Morton Grove, IL). KIC and KC were monitored at 301 AMU and leucine and NL were monitored at 302 AMU by using a gas chromatography-mass spectrometry system (Hewlett-Packard 5970a). Concentrations were calculated by comparing the KIC to KC peak height ratios and leucine to NL peak height ratios with that of the standards.

An automated system (Rotochem IIA, Travenol Laboratories, Deerfield, IL) was used with enzymatic and colorimetric kits for determination of plasma glucose (Glucose (HK) Reagent, Gilford Systems, Oberlin, OH), blood urea nitrogen (BUN; BUN Reagent, Gilford Systems, Oberlin, OH), albumin (SpecTru BCG Albumin Reagent, Pierce Chemical Company, Rockford, IL), and phosphorus (Worthington Inorganic Phosphorus

Reagent Set, Cooper Biomedical, Inc., Malvern, PA). As plasma from Exp. 1 was collected into tubes with EDTA, calcium values from these trials were not reported.

Plasma Hormone Analysis

Plasma was analyzed for cortisol concentration by radioimmunoassay (Amerlex Cortisol Kit, Amersham Corporation, Arlington Heights, IL). Plasma insulin concentration was determined by radioimmunoassay by using bovine insulin antibody (Trenkle, 1972). The concentration of insulin-like growth factor (IGF-I) in plasma was measured by Dr. Ted Elasasser (USDA-ARS, Beltsville, MD) by radioimmunoassay by using human IGF-I antibody (Underwood et al., 1982).

Statistical Analysis

In Exp. 1, statistics were performed by using the general linear model procedure of SAS (SAS, 1985), examining the dose-resposive effect of KIC on the different variables with pen as the experimental unit. The linear and quadratic effects of KIC were tested using an F-test. In Exp. 2, data from trials 1 and 2 were analyzed together and statistics were performed on the various variables with analysis of variance of a replicated model that included diet, experiment and diet x experiment by using the general linear model procedure of SAS (SAS, 1985). F-test with 3 and 47 degrees of freedom was used to test for significance of diet effects, whereas t-test was used to ascertain the probability of differences between treatments.

RESULTS

The effect of orally administered KIC protected from ruminal degradation on cattle performance is reported in Table 4. During the first 30 days of the trial, daily gain tended to be increased by 29%, 17% and 18% for the .02%, .07% and .20% KIC treatments, respectively (probability of a greater F for quadratic-dose response = .20). Daily feed intake was not altered. Feed conversion was improved 13%, 9% and 10% for the .02%, .07% and .20% KIC treatments, respectively (probability of a greater F for quadratic-dose response = .12).

Over the entire 161-d trial, daily gain was increased by KIC in a quadratic dose-responsive manner ($p<.03$). Daily gain was increased by 14% and 7% for the .02% and .07% KIC treatments, respectively. Daily feed intake was not significantly altered. Feed conversion tended to be improved 8% and 6% for the .02% and .07% KIC treatments, respectively (probability for a greater F for quadratic-dose response = .26).

The effect of feeding rumen-protected leucine and its metabolites to lambs on growth, feed intake, feed conversion and wool growth is presented in Table 5. Whereas daily gain was increased 8% and feed conversion was improved 5% for lambs fed KIC, these were not statistically significant ($p<.27$ and $p<.36$, respectively). Daily feed intake and fat-free wool gain were not significantly altered. Leucine and IVA treatment did not significantly alter daily gain, feed intake, feed conversion or wool gain.

In Exp. 1, one steer in the control group died and one steer was treated for sickness, whereas no animals from the KIC-treated groups

Table 4. The effect of feeding rumen-protected α-ketoisocaproate (KIC) on steer growth, intake and feed conversion (Exp. 1)

	---------% Dietary KIC---------					
Parameter	0	.02	.07	.20	SEM[a]	p>F[b]
Starting wt., kg	263	263	263	264	8	
Daily gain, kg						
0-30 days	1.33	1.72	1.55	1.57	.13	.20
0-74 days	1.37	1.56	1.48	1.53	.06	.37
0-128 days	1.27	1.44	1.35		.03	.04
0-161 days	1.21	1.38	1.30		.03	.03
Daily intake, kg DM/day						
0-30 days	6.5	6.9	6.7	6.8	.3	.54
0-72 days	7.8	8.3	8.0	8.0	.4	.56
0-128 days	8.7	9.1	8.7		.3	.45
0-161 days	9.1	9.5	9.1		.4	.40
Feed/gain						
0-30 days	5.0	4.0	4.3	4.4	.3	.12
0-72 days	5.7	5.3	5.4	5.2	.2	.07
0-128 days	6.9	6.3	6.5		.3	.30
0-161 days	7.5	6.9	7.0		.2	.26

[a]SEM is the pooled standard error of the mean.

[b]Probability of a greater F for quadratic-dose response of KIC.

Table 5. The effect of rumen-protected leucine (Leu), α-ketoisocaproate (KIC), or isovalerate (IVA) for approximately 100 days on lamb growth, intake, feed conversion and wool growth

	Control	Leu	KIC	IVA	SEM[a]	P>F[b]	P>F[c]
n	13	12	16	12			
Start weight, kg	23.2	22.6	23.0	22.6	.8	.96	.97
Days to slaughter	101	99	93	98	5	.67	.28
Daily gain, g	282	294	306	295	14	.70	.27
Daily intake, g	1330	1370	1370	1330	40	.89	.53
Feed/gain	4.81	4.78	4.55	4.82	.19	.75	.36
Fat-free wool gain, $mg/(cm^2 \cdot d)$	2.09	1.98	2.09	1.95	.12	.78	.95

[a]SEM is pooled standard error of the mean.

[b]Probability of > F for overall model.

[c]Probability of > F for contrast between control and KIC means.

died or were treated. There was one condemned liver in the control group and one in the 0.07% KIC treatment group. In Exp. 2 Trial 1, two control lambs, one lamb fed leucine and one fed IVA developed urinary calculi or other complications and were removed from the study, whereas, in Exp. 2 Trial 2, one control lamb, one lamb fed leucine and one fed IVA were removed from the study because of urinary calculi and other complications.

The effect of KIC on steer carcass composition is listed in Table 6. Carcass weight was increased by KIC in quadratic dose-responsive manner ($p<.06$). Dressing percentage, longissimus area, backfat thickness, perirenal, pericardial and pelvic fat and yield grade were not affected by the addition of KIC to the diet. However, the number of cattle grading choice or prime was significantly increased in a linear dose-dependent manner by the addition of KIC ($p<.02$). Whereas approximately 70% of the control cattle graded choice or prime, 90% on the .02% and 100% on the .07% KIC treatment graded choice or prime.

The effect of feeding leucine and its metabolites on lamb organ weight is presented in Table 7. The weight of carcass, liver, spleen, heart, kidneys, testis or adrenal glands, expressed as a percentage of body weight, were not altered as result of treatment. Percentage lungs were increased by KIC treatment ($p<.01$).

Carcass composition of lambs fed rumen-protected leucine and its metabolites is presented in Table 8. Fat thickness over the 11th rib was decreased by 17% when KIC treatment was compared with controls ($p<.04$). Perirenal fat and longissimus area were not significantly

Table 6. The effect of feeding rumen-protected α-ketoisocaproate (KIC) for 161 days on steer carcass composition

Parameter	----% Dietary KIC---- 0	.02	.07	SEM[a]	P>F[b]
Final weight, kg	462	485	470	12	.03
Carcass weight, kg	275	290	282	9	.06
Carcass, %[c]	60.0	59.8	59.7	.2	.54
Longissimus area, cm^2	72.2	72.9	72.2	2.3	.94
Backfat thickness, mm	8.3	8.6	7.9	.6	.51
Internal fat, %[d]	2.1	2.1	2.0	.1	.67
Yield grade	1.8	2.0	1.9	.1	.22
Quality grade:					
% Prime and choice[e]	69.7	88.9	100.0	6.1	.02
% Good[f]	29.4	11.1	0.0	6.1	.02
Marbling score[g]	Small 50	Small 90	Modest 10	20	.54

[a]SEM is the pooled standard error of the mean.
[b]Probability of a greater F for linear-dose response of KIC, except carcass weight and yield grade, which were for quadratic-dose response.
[c]Percentage of final body weight.
[d]Perirenal, pericardial and pelvic fat as a percentage of final body weight.
[e]Percentage of steers grading prime and choice.
[f]Percentage of steers grading good.
[g]Marbling score graded in units of Slight 10, 20, ... 90; Small 10, 20, ... 90; Modest 10, 20, ... 90; Moderate 10, 20, ... 90. SEM in units of 10.

Table 7. The effect of feeding rumen-protected leucine (Leu), α-ketoisocaproate (KIC) or isovalerate (IVA) for approximately 100 days on lamb organ weight

	Control	Leu	KIC	IVA	SEM[a]	P>F[b]	P>F[c]
Final weight, kg	50.7	50.9	51.0	50.9	.6	.98	.68
Carcass, %[d]	52.1	52.1	53.1	52.8	.7	.65	.29
Liver, %[d]	1.68	1.82	1.79	1.73	.05	.37	.22
Spleen, %[d]	.213	.208	.228	.185	.011	.08	.31
Heart, %[d]	.41	.42	.42	.42	.01	.75	.32
Lungs, %[d]	.90	1.01	1.10	.96	.05	.07	.01
Kidney, %[d]	.28	.31	.30	.30	.01	.33	.22
Testis, %[d]	.47	.51	.44	.46	.03	.37	.46
Adrenal glands, %[d] X 1000	4.99	4.96	5.18	5.01	.08	.94	.63

[a]SEM is pooled standard error of the mean.

[b]Probability of > F for overall model.

[c]Probability of > F for contrast between control and KIC means.

[d]Percentage of final body weight.

Table 8. The effect of feeding rumen-protected leucine (Leu), α-ketoisocaproate (KIC) or isovalerate (IVA) for approximately 100 days on lamb carcass composition

	Control	Leu	KIC	IVA	SEM[a]	P>F[b]	P>F[c]
Backfat, mm	7.0	6.8	5.8	6.4	.5	.19	.04
Perirenal fat, %[d]	1.53	1.28	1.26	1.44	.16	.57	.22
Longissimus area, cm^2	2.69	2.63	2.61	2.56	.12	.83	.51
Psoas major, %[d]	.284	.282	.314	.300	.012	.16	.07
Longissimus, %[d]	.788	.807	.849	.793	.022	.18	.05
Gastrocnemius, %[d]	.111	.117	.124	.107	.004	.08	.07
Tibia, %[d]	.257	.271	.269	.250	.006[e]	.03	.09
Humerus, %[d]	.145	.160	.164	.147	.003[f]	.01	.006
Tibia, cm	20.7	21.0	20.8	20.7	.2	.69	.51
Humerus, cm	13.3	13.7	13.5	13.4	.2	.48	.90
Tibia, g/cm	6.30	6.11	6.60	6.11	.13	.05	.09
Humerus, g/cm	5.21	5.64	5.79	5.28	.14	.05	.04

[a]SEM is pooled standard error of the mean.

[b]Probability of > F for overall model.

[c]Probability of > F for contrast between control and KIC means.

[d]Percentage of final body weight.

[e]Probability of > F for contrast between control and leucine means = .08.

[f]Probability of > F for contrast between control and leucine means = .06.

affected by treatment. However, KIC increased psoas, longissimus and gastrocnemius muscle weights by 11% ($p<.07$), 8% ($p<.05$) and 12% ($p<.07$), whereas leucine and IVA did not significantly affect these parameters.

KIC and leucine both increased tibia weight as a percentage of body weight 5% ($p<.09$ and $p<.08$, respectively) and increased humerus weight 13% ($p<.006$) and 10% ($p<.06$), respectively. IVA did not alter tibia or humerus weight. The length of these bones were not altered due to treatment. The weight per length of the tibia was increased 5% ($p<.09$) and 4% ($p<.17$) for KIC and leucine treatments, respectively. The weight per length of humerus was increased 11% ($p<.04$) and 8% ($p<.19$) for the KIC and leucine treatments, respectively. IVA did not affect the weight per length in either bone.

The effect of KIC on steer plasma parameters is reported in Table 9. Plasma glucose concentration was decreased in a linear dose-responsive manner ($p<.04$). KIC fed at .02% and .07% of the diet decreased glucose 16% and 19%, respectively. Plasma albumin, BUN and phosphous concentrations were not affected by treatment.

Cattle cortisol concentrations were decreased quadratically ($p<.02$) by 19% and 14% for the .02% and .07% KIC treatments, respectively. Plasma IGF-I and insulin concentrations were not altered. However, glucagon concentrations were decreased and consequently insulin/glucagon were increased in a linear dose-responsive manner ($p<.09$ and $p<.06$, respectively).

The effect of leucine and its metabolites fed to lambs on plasma parameters is presented in Table 10. Although leucine and IVA did not

Table 9. The effect of feeding rumen-protected α-ketoisocaproate (KIC) for 72 days on steer plasma parameters

Parameter	----% Dietary KIC---- 0	.02	.07	SEM[a]	P>F[b]
KIC, uM	20.4	22.4	19.8	2.7	.76
Leucine, uM	256	233	240	19	.72
Glucose, mg/dl	116	97	94	6	.04
BUN, mg/dl	13.0	13.7	14.0	.6	.22
Albumin, mg/dl	3.99	4.05	3.93	.1	.71
Phosphorus, mg/dl	10.1	10.0	10.1	.5	.99
Cortisol, ng/dl	3.47	2.80	2.99	.11	.02
IGF-I, ng/ml	120	113	118	12	.96
Insulin, ng/ml	1.30	1.14	1.20	.21	.83
Glucagon, pg/ml	1428	818	697	219	.09
Insulin/glucagon	.99	1.41	1.74	.21	.06

[a]SEM is the pooled standard error of the mean.

[b]Probability of a greater F for linear-dose response of KIC with the exception of cortisol, which is for a quadratic-dose response.

Table 10. The effect of feeding rumen-protected leucine (Leu), α-ketoisocaproate (KIC) or isovalerate (IVA) for approximately 28 days on lamb serum parameters

	Control	Leu	KIC	IVA	SEM[a]	P>F[b]	P>F[c]
KIC, uM	22.7	21.2	19.5	20.9	1.8	.63	.20
Leucine, uM	217	228	218	248	17	.58	.99
Glucose, mg/dl	89	85	90	88	2	.55	.81
Albumin, mg/dl	4.05	3.82	4.02	4.02	.08	.20[e]	.87
BUN, mg/dl	21.5	22.1	23.8	23.4	1.2	.40	.12
Calcium, mg/dl	10.7	10.3	10.7	10.7	.1	.23[f]	.98
Phosphorus, mg/dl	9.3	9.0	10.2	9.8	.3	.04	.04
Cortisol, ug/dl	2.31	3.17	3.65	2.59	.46	.14	.03
IGF-I, ng/ml[d]	203	212	220	201	15	.82	.57
Insulin, ng/ml	1.47	1.79	1.63	1.65	.35	.99	.76
Glucagon, pg/ml[d]	326	321	295	295	16	.40	.19
Insulin/glucagon[d]	2.68	3.33	3.43	2.56	.42	.19	.22

[a]SEM is pooled standard error of the mean.

[b]Probability of > F for overall model.

[c]Probability of > F for contrast between control and KIC means.

[d]Values from trial 1 only.

[e]Probability of > F for contrast between control and leucine means = .06.

[f]Probability of > F for contrast between control and leucine means = .11.

alter plasma leucine and KIC concentrations, KIC treatment did result in a lowered level of plasma KIC ($p<.06$). Glucose, albumin and BUN levels were not altered due to leucine, KIC or IVA, with the exception that leucine decreased plasma albumin ($p<.06$). Whereas KIC and IVA did not alter plasma calcium, there was a trend for leucine to decrease calcium ($p<.11$). Phosphorus levels were increased by KIC 10% ($p<.04$) but were not altered by leucine or IVA treatment.

In Exp. 2, plasma cortisol was increased by KIC ($p<.03$), whereas leucine and IVA had no affect. IGF-I, insulin, glucagon and insulin/glucagon were not altered by leucine or its metabolites.

Discussion

Oral administration of ruminally-protected KIC altered steer and lamb growth rates as effectively as larger doses delivered intraperitoneally or orally in an unprotected form. Cattle responded to KIC supplementation early in the experiment, with the greatest response being 29% at 30 days with .02% KIC. Although response to KIC was greater early in the trial, a significant response was maintained over the duration of the trial. The 0.02% KIC treatment continued to provide the greatest response (14% increase) throughout the trial.

In Exp. 2, the lambs also had greater response to KIC early in the trials (23% at 28 days, $p<.15$; data not shown). Over the duration of the trial, there was a trend for KIC (5 g/d) to increase gain 8% and feed conversion 5%. These data are consistent with results from previous trials (Flakoll and Nissen, 1986; Flakoll et al., 1986) in

which, when KIC was administered intraperitoneally (3 g/d), gain was increased 11% and, when KIC was administered unprotected orally (15 g/d), gain was increased 10%.

Cattle subcutaneous and internal fat depots were not altered by KIC, whereas these fat depots in lambs were decreased 17 and 18%, respectively. This fat decrease in lambs is similar to previous trials where intraperitoneal KIC decreased subcutaneous fat 28% and perirenal fat 13% (Flakoll and Nissen, 1986,) and oral KIC decreased subcutaneous fat 18% and intraperitoneal fat 14% (Flakoll et al., 1986).

The reason for the differences between the cattle and lamb experiments may lie in the differences in how the experiments were terminated. Lambs were killed at the same final weight, whereas the cattle were killed at the same time, resulting in different final weights between treatments. It could be postulated that, if the control cattle were fed to an equivalent final weight, they would have had more marbling and a greater percentage would have graded choice or prime. However, it is likely that feeding the control animals to an increased weight would have resulted in increased subcutaneous and internal fat depots and worsened yield grades and feed converisons.

In addition to decreasing internal and external fat depots in lambs, KIC increased muscle weight. The most likely mechanism whereby this would occur would be through its proposed effects on decreasing protein degradation (Chua et al., 1979; Poso et al., 1982; Tischler et al., 1982) or increasing protein synthesis (Kirsch et al., 1976). Additionally, KIC increased insulin to glucagon ratios in both

experiments, which would tend to decrease protein turnover and favor increased protein deposition. KIC also decreased the catabolic hormone cortisol in the cattle experiment, which should favor increased gain. In lambs, however, KIC increased cortisol concentrations. The reason for these opposing effects is unclear.

KIC and leucine increased tibia and humerus weights as percentage of the body weight. This is similar to a previous study where intraperitoneal KIC increased percent bone and bone density (Flakoll and Nissen, 1986; Flakoll et al., 1986). Both leucine and KIC reduced plasma calcium to phosphorus ratios. Whereas leucine accomplished this by reducing plasma calcium, KIC increased plasma phosphorus. Previous studies with KIC have demonstrated decreased plasma phosphorus and increased calcium to phosphorus (Walser, 1975; Heidland et al., 1978; Frohling et al., 1980; Kampf et al., 1980; Attman et al., 1984). The alteration of phosphorus metabolism may involve the reduction of parathyroid hormone concentrations, which have been reported to decrease during ketoacid therapy (DiLandro et al., 1982; Schmicker et al., 1982; Lindenau et al., 1982; Barsotti et al., 1982). IGF-I is also known to alter bone metabolism. However, plasma IGF-I concentrations were not altered in these studies.

It should be noted that animals in these experiments were on a high plane of nutrition. This was done to ensure that animals would be supplied with a slight excess of nutrients to allow for increased growth. It could be argued, however, that KIC may be more effective when nutrients are limiting, as it has been shown that KIC improves

nitrogen balance in fasting humans (Sapir and Walser, 1977). Further research is required to define nutrient interaction with KIC treatment.

Feeding leucine and IVA did not significantly affect growth or fat and muscle deposition. This may possibly be explained by the physiological differences in the metabolism between the different leucine metabolites. Whereas leucine, KIC and IVA share the same metabolic pathway, their site of metabolism is distinctly different. The liver is limiting in branched chain amino acid transaminase, so very little dietary leucine is catabolized until it reaches extrahepatic tissues (Ichihara and Koyama, 1966; Krebs and Lund, 1977). Conversely, the liver contains abundant quantities of branched chain ketoacid dehydrogenase (Shinnick and Harper, 1976), which is the enzyme responsible for the first irreversible step of KIC oxidation in which KIC is converted to isovaleryl-CoA. Therefore, an oral dose of leucine would be expected to undergo very little catabolism by the splanchnic tissues. It has been estimated that 70 to 95% of an oral branched chain amino acid load escapes the gut and liver tissues (Elwyn et al., 1968; Bloomgarden et al., 1981). In contrast, KIC would be expected to be extensively catabolized while passing through the splanchnic area. In dogs, it has been demonstrated that 85% of an oral dose of KIC is metabolized by the splanchnic tissues (Abumrad et al., 1982; Bassler and Pietrek, 1983).

IVA would be expected to be metabolized in the same sites as KIC. However, IVA cannot be converted back to KIC. Therefore, if IVA effects mimicked KIC, it could be assumed that KIC acted through IVA. However,

this was not the case in the present experiments, because IVA did not produce similar effects to KIC.

It was noted that the plasma concentrations of glucose, cortisol, and glucagon in the cattle experiment were above that which would normally be expected, indicating that the animals may have been under stress. This may have been the case as each animal was placed in a mechanical squeeze-shoot, immobilized with a halter and blood sampled via venipuncture. Additionally, the weather during this period was wet and cold (~5^{o} C). The reported lowering of stress indicators cortisol and glucagon may provide insight into another possible mechanism for KIC. KIC could suppress the catabolic action of stress hormones, resulting in increased gain. This may explain the observation of increased response to KIC early in the experiments when stress is thought to be greater. Additional support for this mechanism is found in studies with lambs and cattle where lymphocyte blastogenesis and antibody production are increased with KIC treatment (Nissen et al. 1986; Flakoll et al., 1987; Kuhlman et al., 1987).

In conclusion, while the optimal dose may differ for various metabolic effects, supplemental KIC protected from ruminal degradation has the potential to improve growth and alter energy and protein partitioning in cattle, whereas leucine and IVA did not produce similar responses.

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Muscle growth and whole-body leucine, phenylalanine and 3-methylhistidine metabolism of growing lambs fed leucine, α-ketoisocaproate and isovalerate protected from ruminal degradation[1]

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[1] Project 2614. This work was supported in part by NIH Grant AM32540 and the Iowa Agriculture and Home Economics Experiment Station, Ames.

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[3]Appreciation is expressed to S. Feurbach, J. Cain, S. Rogers, T. Kuan, C. Telleen, B. Beer and J. Huebsch for their able technical assistance. We are grateful to Dr. T. Elsasser at the USDA-ARS, Beltsville, MD, for measuring plasma IGF-I concentrations.

SECTION III. MUSCLE GROWTH AND WHOLE-BODY LEUCINE, PHENYLALANINE AND 3-METHYLHISTIDINE METABOLISM OF GROWING LAMBS FED LEUCINE, α-KETOISOCAPROATE AND ISOVALERATE PROTECTED FROM RUMINAL DEGRADATION

Abstract

Leucine (Leu; n=6) and its metabolites, α-ketoisocaproate (KIC; n=8), isovalerate (IVA; n=6) and limestone (control; n=6) were fed in a ruminally-protected form at 0.05% of the diet to growing ram lambs to determine effects on whole-body Leu, KIC, phenylalanine (Phe), tyrosine (Tyr) and 3-methylhistidine (3MH) metabolism by using primed-dose constants infusion of [6,6,6-$^{2}H_{3}$] Leu, [1-^{13}C] KIC, [1-^{13}C] Phe and 3-methyl-$^{2}H_{3}$-histidine. Subcutaneous fat thickness was decreased 24% ($p<.08$) and 23% ($p<.09$) by KIC and IVA, respectively. Perirenal fat depots were decreased 44% ($p<.005$) and 27% ($p<.07$) by KIC and Leu, respectively. KIC increased psoas major, longissimus and gastrocnemius muscle weight by 13% ($p<.06$), 8% ($p<.15$) and 9% ($p<.13$), respectively. Leu used for protein synthesis (avg 4.42 umol/(min*kg)), Leu from proteolysis and absorption (avg 4.66 umol/(min*kg)), KIC transamination to Leu (avg .62 umol/(min*kg)), KIC oxidation (avg .22 umol/(min*kg)), Phe flux (avg 1.29 umol/(min*kg)), Tyr derived from Phe (avg 51.8%) and 3MH flux (avg 12.0 nmol/(min*kg)) were not significantly altered by dietary treatment. Leu transamination to KIC (avg .83 umol/(min*kg)) was increased for the Leu-treated lambs ($p<.06$). In conclusion, although KIC increased muscle mass, it does not seem to occur through protein-sparing.

(Keywords: Lambs, Leucine, α-Ketoisocaproate, Muscle growth, Phenylalanine, 3-Methylhistidine.)

INTRODUCTION

Initial experiments have shown that α-ketoisocaproate (KIC) can improve lamb and cattle growth. When lambs were injected intraperitoneally with 3 g KIC daily, growth was improved 11% (Flakoll et al., 1988a), whereas lambs fed approximately 15 g KIC daily had increased gains of 10% (Flakoll et al., 1988a). KIC protected from ruminal degradation increased growth 8% when fed to lambs at approximately .75 g daily (Flakoll et al., 1988b). Steers fed approximately 1.3 g KIC protected from ruminal degradation had gain increased by 14% (Flakoll et al., 1988b). In addition to effects on growth, KIC was demonstrated to increase milk and milk fat production in lactating cattle 10 and 8%, respectively (VandeHaar et al., 1988).

In each of the lamb trials, internal and external fat depots were decreased (Flakoll et al., 1988a,b), whereas psoas, gastrocnemius and longissimus muscles dissected from lambs fed KIC protected from ruminal degradation were approximately 10% larger than that of the controls (Flakoll et al., 1988b). These data suggest that some aspect of muscle protein metabolism is altered by KIC. These findings in ruminants are supported by data in human studies in which nitrogen balance was improved by KIC treatment during fasting (Mitch et al., 1981), postsurgical trauma (Sapir et al., 1983) and renal failure (Ell et al., 1978).

Metabolites of KIC, leucine and isovalerate (IVA), have also been proposed to be important in regulation of protein metabolism. Leucine has been found to increase protein synthesis (Morgan et al., 1971; Buse

and Reid, 1975), whereas IVA has been reported to decrease proteolysis (Chua et al., 1979).

Therefore, the present experiment was designed to determine if leucine metabolites affect whole-body protein metabolism by measuring the flux of leucine and an amino acid unrelated to leucine, phenylalanine. Additionally, possible effects of leucine metabolites on muscle proteolysis was determined by measuring the flux of 3-methylhistidine.

MATERIALS AND METHODS

Animals and Diets

Thirty mixed-breed ram lambs, weighing 21 kg, were randomly allocated to one of four dietary treatment groups: 1) leucine, 2) KIC, 3) IVA or 4) limestone (control). All animals were fed a corn-soybean meal basal diet (Table 1) supplemented with 5 g ammonium chloride daily. The lambs were caged individually in a light- and temperature-controlled environment with free access to water.

Dietary treatments consisted of leucine (n=7), KIC (n=8), IVA (n=7) or limestone (control; n=8), which were supplemented to the basal diet twice daily as zein-coated pellets (Table 2). Lambs were fed their respective pellets at a rate of 1 g/d for the first 6 wk, 1.5 g/d for wk 6-10 and 2 g/d for wk 10 to time of slaughter. These doses translate to about 1 g pellets per kg diet, which is equivalent to 2.5 mmol leucine, KIC and IVA per kg diet or about .05% leucine added to the diet.

Pellets were made by thoroughly mixing zein and 88% ethanol and adding limestone, bentonite and either $Ca(KIC)_2$, $Ca(IVA)_2$, leucine or more limestone. This mixture was extruded through a 3-mm die directly into a vat of liquid nitrogen for freeze-fracturing. The frozen, shattered pellets were allowed to dry slowly at room temperature. After drying, the pellets were shaken under forced air and sprayed with a solution of 20% zein in 88% ethanol (100 ml/kg pellets). Average pellet size was 2 x 8 mm and density was 1.1 -1.2 g/ml. Ruminal inoculation of KIC in Dacron bags indicated that ruminal bypass of the

Table 1. Composition of basal diet for lambs

Ingredient	% of total mix (as fed basis)
Corn grain	60
Expeller soybean meal[a]	21
Dehydrated alfalfa meal[b]	10
Molasses, sugarcane	7.5
Corn oil[c]	.38
Limestone	.50
Salt	.50
Vitamin A premix[d]	.10
Trace mineral premix[e]	.02
Calculated:	
Dry matter, %	88
Crude protein, %[f]	17
Metabolizable protein, %[g]	124
Leucine, %[f]	1.8
Net energy- maintenance, Mcal/kg[f]	1.9
Net energy- gain, Mcal/kg[f]	1.3

[a]Soy-Plus, West Central Cooperative, Ralston, IA.
[b]20% crude protein.
[c]Mazola, CPC International., Englewood Cliffs, NJ.
[d]Ca, 13-15%; Zn 12%; Mn 8.0%; Fe 10%; Cu 1.5%; I .2%; Co .1%; Calcium Carbonate division of J. M. Huber Corporation, Quincy, IL.
[e]5,200,000 IU Vitamin A/kg, Hoffmann-LaRoche, Nutley, NJ.
[f]Calculated from National Research values (1982).
[g]Calculated from Burroughs et al. (1974).

Table 2. Composition of treatment supplements[a]

Ingredient	Leucine	KIC	IVA	Control
	----------g/kg----------			
Zein[b]	450	450	450	450
Ethanol	278	278	278	278
Water	37	37	37	37
Limestone,[c]	16	10	8	45
Sodium Bentonite,[d]	5	5	5	10
Leucine[e]	340			
α-Ketoisocaproate[f]		400		
Isovalerate[g]			420	

[a]Dried treatment pellets contained approximately 2.5 mol leucine, KIC, or IVA, and 64, 91, 97, or 180 g calcium per kg leucine, KIC, IVA, or control pellets, respectively.

[b]Freeman Industries, Tuckahoe, NY.

[c]325 mesh; Iowa Limestone Company, Des Moines, IA.

[d]200 mesh; American Colloid Company, West Des Moines, IA.

[e]Sigma Chemical Company, St. Louis, MO.

[f]Ca (α-ketoisocaproate)$_2$ H_2O; SOBAC, Rue Saint-Honore, 75001, Paris, France.

[g]Ca (isovalerate)$_2$ H_2O prepared from isovaleric acid (Sigma Chemical Company, St. Louis, MO) as described in Materials and Methods.

compounds was approximately 60% (VandeHaar et al., 1987). $Ca(IVA)_2$ was made by combining calcium hydroxide with IVA.

Lambs were weighed weekly and total feed consumption for each two week period was recorded. During the study, two control lambs, one lamb fed leucine and one fed IVA developed urinary calculi or other complications and were removed from the experiment.

When animals weighed approximately 50 kg, they were fasted 18 hr and electrically stunned and exsanguinated. Perirenal fat, left and right gastrocnemius muscles, left and right psoas major muscles, and left and right longissimus dorsi muscles (posterior to tenth rib) were separated and weighed. Carcasses were severed between the tenth and eleventh ribs; backfat thickness and longissimus area were measured.

Isotope Infusion Study

During wk 8, when lamb average body weight was 40 kg, 4 lambs daily were infused with stable isotopes to study the flux of leucine, KIC, phenylalanine and 3-methylhistidine. On the day before each study, lambs were removed from feed at 2000 hr and placed in metabolism stalls. At 0730 hr on the day of study, lambs were fed 50 g of feed with .75 g of their respective treatment pellets. Both the right and left jugular veins were catheterized, and at about 1000 hr a primed dose-continuous infusion of [6,6,6-$^{2}H_3$] leucine, [1-^{13}C] KIC, [1-^{13}C] phenylalanine and 3-methyl-$^{2}H_3$-histidine was begun into the right jugular vein (Table 3).

After a 120-min equilibration period, blood was sampled from the left jugular vein every 15 min from 120 min to 180 min. Blood was

Table 3. Infusion rate of isotopes

	Priming dose (mg)[a]	Steady state dose (mg/min)
[6,6,6-$^{2}H_3$] L-Leucine[b]	14.1	0.364
[1-^{13}C] L-Phenylalanine[c]	12.1	0.312
[1-^{13}C] 2-Ketoisocaproate[c] (sodium salt)	14.1	0.364
3-Methyl-$^{2}H_3$-L-histidine[b]	0.113	0.00291

[a]Infused over the first 10 min.

[b]MSD Isotopes, St. Louis, MO.

[c]Tracer Technologies, Inc., Newton, MA.

collected into sodium EDTA-coated tubes and immediately put on ice until centrifugation; plasma was then stored at -70°C.

Plasma Metabolite Analysis

Concentrations of plasma leucine, KIC, phenylalanine and tyrosine were analyzed by adding 20 nmol -ketocaproate (KC) and 150 nmol norleucine (NL) as internal standard to 1 ml plasma. After deproteinizing with 3 ml .75 N perchlorate, the amino and keto acids were separated via cation exchange column. The keto acids were collected into 35 ml of methylene chloride and back extracted with .5 ml of .05 N NaOH (Nissen et al., 1982). The amino acids were eluted from the column with 4 ml 25% NH_4OH. Keto and amino acid extracts were dried under nitrogen gas with 55° C heat and derivatized with 100 ul pyridine plus 100 ul N-methyl-N (t-butyl-dimethylsilyl) trifluoroacetamide containing 1% t-butyldimethylchlorosilane (Regis Chemical Company, Morton Grove, IL). Using a gas chromatography-mass spectrometry system (Hewlett-Packard 5890/5970a), the keto acids, KIC and KC, were monitored at 301.2, 302.2, 303.2 and 304.2 AMU to determine m+1 (^{13}C-KIC) and m+3 ($^{2}H_3$-KIC) enrichments of KIC, whereas the amino acids, leucine and NL, were monitored at 302.2, 303.2, 304.2 and 305.2 AMU to determine m+1 (^{13}C-leucine) and m+3 ($^{2}H_3$-leucine) enrichments of leucine; phenylalanine and tyrosine were monitored at 302.2 and 303.2 to determine the m+1 (^{13}C-phenylalanine and ^{13}C-tyrosine) enrichments of phenylalanine and tyrosine. Concentrations of each in plasma were calculated by comparing the KIC-to-KC ratio and leucine-, phenylalanine-

Table 4.

Plasma 3-methylhistidine was analyzed by adding 177 nmol of 1-methylhistidine as an internal standard to 2 ml of plasma. After deproteinizing with 3 ml .75 N perchlorate, the supernatant was poured over a cation exchange column, and 3-methylhistidine was eluted from the column with 4 ml 25% NH_4OH and dried under nitrogen gas with 55° C heat. Additional heat (100° C for 15 min) was provided to volatilize any remaining hydroscopic water. After cooling, 100 ul acetonitrile plus 100 ul N-methyl-N (t-butyl-dimethylsilyl) trifluoroacetamide (without 1% t-butyldimethyl-chlorosilane; Regis Chemical Company, Morton Grove, IL) was added and heated at 70° C for derivatization. After derivatization, a gas chromatograhy-mass spectrometry system (Hewlett-Packard 5890/5970a) was used to detect 3-methylhistidine at 238.1, 239.1, 240.1 and 241.1 AMU and determine m+3 (3-methyl-$^2H_3$-histidine) enrichment of 3-methylhistidine. Concentration of plasma 3-methylhistidine was determined by comparing the peak height ratios of 3-methylhistidine to 1-methylhistidine in standards versus plasma. Column temperatures and retention times are included in Table 4. Observed spectra of 3-methylhistidine are illustrated in Figure 1.

Enrichments of ^{13}C-KIC, $^2H_3$-KIC, ^{13}C-leucine and $^2H_3$-leucine were used to determine the whole-body flux of leucine and KIC, and estimate leucine to protein synthesis, leucine entry rate (from proteolysis and absorption), leucine and KIC interconversion and KIC oxidation according to the equations of Nissen and Haymond (1981). Whole-body phenylalanine flux was estimated by the equation: phenylalanine flux = infusion rate

Table 4. Parameters for gas chromatography-mass spectroscopy analysis of leucine (Leu), phenylalanine (Phe), tyrosine (Tyr), norleucine (NL), α-ketoisocaproate (KIC), α-ketocaproate (KC), 3-methylhistidine (3MH) and 1-methylhistidine (1MH)[a]

Parameter	Leu, Phe, Tyr, NL[b]	KIC, KC[b]	3MH, 1MH[c]
Temperatures, °C			
Injector	285	285	285
Detector	285	285	285
Oven-initial	100, .5 min	100, .5 min	50, .5 min
-ramp 1	40/min to 220 Hold 2 min	40/min to 220 Hold 2 min	40/min to 220 Hold 5 min
-ramp 2	60/min to 300 Hold 1.2 min	60/min to 300 Hold 1.2 min	40/min to 300 Hold 1.3 min
Retention times, min			
Leu	3.8		
NL	4.0		
Phe	5.3		
Tyr	7.0		
KIC		3.9	
KC		4.1	
3MH			7.8
1MH			8.5
Ions monitored, AMU	302.2, 303.2 304.2, 305.2	301.2, 302.2 303.2, 304.2	238.1, 239.1 240.1, 241.1

[a]Hewlett-Packard 5890 gas chromatograph and 5790a mass selective detector using splitless injection with purge off for .4 min and a 25 m long x .22 mm i.d. x .11 um film thickness, crosslinked silicon gum phase capillary column (HP-1, Hewlett-Packard, Avondale, PN).

[b]Formed di-derivative with N-methyl-N (t-butyl-dimethylsilyl) trifluoroacetamide containing 1% t-butyldimethylchlorosilane (Regis Chemical Company, Morton, IL).

[c]Formed di-derivative with N-methyl-N (t-butyl-dimethylsilyl) trifluoroacetamide without 1% t-butyldimethylchlorosilane (Regis Chemical Company, Morton, IL).

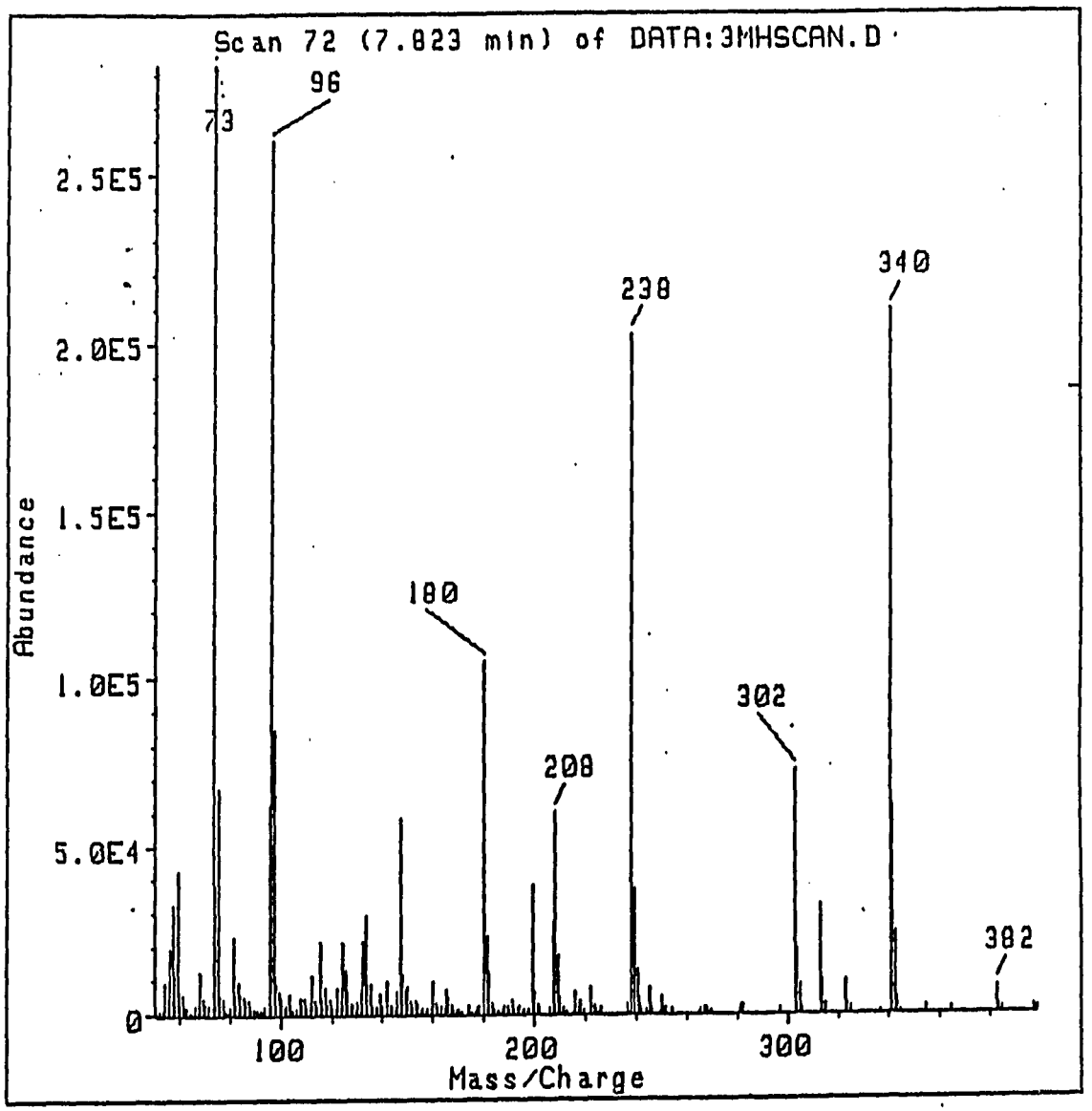

Figure 1. Spectral scan of 3-methylhistidine on a Hewlett-Packard 5790a mass selective detector

flux was estimated by the equation: phenylalanine flux = infusion rate of ^{13}C-phenylalanine / ^{13}C-phenylalanine enrichment. The percentage of whole-body tyrosine from phenylalanine was calculated by the equation: % tyrosine from phenylalanine = (^{13}C-tyrosine enrichment/ ^{13}C-phenylalanine enrichment) X 100. Flux of 3-methylhistidine was extimated by the equation: 3-methylhistidine flux = infusion rate of 3-methyl-$^{2}H_3$-histidine / 3-methyl-$^{2}H_3$-histidine enrichment.

Plasma Hormone Analysis

Plasma was analyzed for cortisol concentration by radioimmunoassay (Amerlex Cortisol Kit, Amersham Corporation, Arlington Heights, IL). Plasma insulin concentrations were determined by radioimmunoassay by using bovine insulin antibody (Trenkle, 1972). Plasma glucagon was also determined by radioimmunoassay by using rabbit anti-porcine glucagon antibody (BioTek Research, Inc., Lenexa, KS). The concentration of insulin-like growth factor (IGF-I) in plasma was measured by Dr. Ted Elasasser (USDA-ARS, Beltsville, MD) by radioimmunoassay using human IGF-I antibody (Underwood et al., 1982).

Statisical Analysis

Data were statistically analyzed by analysis of variance. An F-test with 3 and 22 degrees of freedom was used to test for diet effects and t-test was used to ascertain the probability of differences between treatments. Pearson correlation coefficients were calculated between the variables by SAS procedures (1985).

RESULTS

Growth and feed conversion of the lambs fed leucine and its metabolites are presented in Table 5. Daily gain was not significantly different between control, KIC or IVA treatments. Leucine, however, tended to decrease gain (11%, $p<.13$) when compared to that of controls. Leucine-treated animals had 14% lower gain than did KIC-treated animals ($p<.05$). Feed conversion was not altered by leucine and IVA when compared to that of controls, whereas KIC improved feed conversion 6% over the controls ($p<.19$) and 10% over leucine ($p<.03$).

The effects of feeding leucine and its metabolites on carcass composition are reported in Table 6. Subcutaneous backfat thickness was decreased 24% ($p<.08$) and 23% ($p<.09$) by KIC and IVA, respectively. Perirenal fat depots were decreased 44% ($p<.005$) and 27% ($p<.07$) by KIC and leucine, respectively. KIC increased psoas, longissimus and gastrocnemius muscle weight by 13% ($p<.06$), 8% ($p<.15$) and 9% ($p<.13$), respectively. IVA and leucine did not significantly alter muscle weight.

Plasma metabolites of the lambs fed leucine, KIC or IVA are reported in Table 7. Concentrations of leucine, phenylalanine and tyrosine were not altered as a result of treatments imposed. Plasma KIC concentration was not altered by leucine and IVA but was reduced 28% by KIC treatment ($p<.06$).

The effect of feeding leucine, KIC and IVA to lambs on plasma hormone concentrations are reported in Table 8. Plasma cortisol, IGF-I, insulin and glucagon concentrations were not different between controls

Table 5. The effect of rumen-protected leucine (Leu), α-ketoisocaproate (KIC) or isovalerate (IVA) fed for approximately 100 days on lamb growth and feed conversion

	Control	Leu	KIC	IVA	SEM[b]	--Probability for > F[a]- Overall Model	--Control vs--- Leu	KIC	IVA
n	6	6	8	6					
Daily gain, g	323	288	334	315	16	.22[c]	.13	.63	.74
Feed/gain	4.47	4.66	4.19	4.42	.14	.19[d]	.61	.19	.81

[a]Probability of > F for the overall ANOVA model, the contrast between the control vs Leu means, the contrast between the control vs KIC means, and the contrast between the control vs IVA means.

[b]SEM is pooled standard error of the mean.

[c]Probability of > F for the contrast between the KIC and Leu treatment means = .05.

[d]Probability of > F for the contrast between the KIC and Leu treatment means = .03.

Table 6. The effect of feeding rumen-protected leucine (Leu), α-ketoisocaproate (KIC) or isovalerate (IVA) for approximately 100 days on lamb carcass composition

	Control	Leu	KIC	IVA	SEM[b]	--Probability for > F[a]- Overall Model	--Control vs--- Leu	KIC	IVA
Final weight, kg	50.7	49.9	50.2	50.3	.7	.98	.54	.66	.54
Backfat, mm	9.5	8.6	7.2	7.3	.9	.23	.53	.08	.09
Renal fat, %[c]	1.56	1.14	0.87	1.33	.16	.04	.07	.005	.31
Psoas major, %[c]	.385	.400	.434	.412	.018	.29	.56	.06	.29
Longissimus, %[c]	.885	.865	.959	.913	.035	.29[d]	.70	.15	.58
Gastrocnemius, %[c]	.159	.164	.174	.152	.007	.17[e]	.60	.13	.50

[a]Probability of > F for the overall ANOVA model, the contrast between the control vs Leu means, the contrast between the control vs KIC means, and the contrast between the control vs IVA means.

[b]SEM is pooled standard error of the mean.

[c]Percentage of final body weight.

[d]Probability of > F for the contrast between the Leu and KIC treatment means = .07.

[e]Probability of > F for the contrast between the KIC and IVA treatment means = .03.

Table 7. The effect of feeding rumen-protected leucine (Leu), α-ketoisocaproate (KIC) or isovalerate (IVA) for approximately 100 days on lamb plasma metabolites

	Control	Leu	KIC	IVA	SEM[b]	--Probability for > F[a]-- Overall Model	--Control vs--- Leu	KIC	IVA
KIC, uM	15.2	13.2	11.0	14.0	1.5	.27	.64	.06	.58
Leucine, uM	242	254	215	247	31	.60	.80	.55	.92
Phenylalanine, uM	19.7	16.6	21.0	22.2	2.1	.31	.58	.68	.32
Tyrosine, uM	46.5	39.0	43.9	43.3	6.8	.97	.55	.78	.75
3-Methylhistidine[c] uM	96.3	98.0	112.0	86.0	9.6	.39	.90	.30	.51

[a]Probability of > F for the overall ANOVA model, the contrast between the control vs Leu means, the contrast between the control vs KIC means, and the contrast between the control vs IVA means.

[b]SEM is pooled standard error of the mean.

[c]Only 10 lambs (control-2, Leu-3, KIC-3 and IVA-2).

Table 8. The effect of feeding rumen-protected leucine (Leu), α-ketoisocaproate (KIC) or isovalerate (IVA) fed for approximately 100 days on lamb plasma hormones

	Control	Leu	KIC	IVA	SEM[b]	--Probability for > F[a]- Overall Model	--Control vs--- Leu	KIC	IVA
Cortisol, ug/dl	1.69	2.66	2.20	2.30	.48	.58	.17	.70	.61
IGF-I, ng/ml	203	212	220	201	15	.82	.67	.57	.93
Insulin, ng/ml	.87	.73	.99	.98	.12	.40[c]	.56	.51	.50
Glucagon, pg/ml	326	321	295	295	16	.40	.81	.19	.18
Insulin /glucagon	2.68	2.26	3.43	3.33	.42	.19[d]	.50	.22	.30

[a]Probability of > F for the overall ANOVA model, the contrast between the control vs Leu means, the contrast between the control vs KIC means, and the contrast between the control vs IVA means.

[b]SEM is pooled standard error of the mean.

[c]Probability of > F for the contrast between the Leu and KIC treatment means = .14.

[d]Probability of > F for the contrast between the Leu and KIC treatment means = .06.

leucine, KIC and IVA treatments, although KIC and IVA tended to lower glucagon ($p<.19$ and $p<.18$, respectively). In addition, KIC tended to increase insulin/glucagon 28% ($p<.22$) over that of the control and 51% over the leucine treatment ($p<.06$).

Whole-body leucine, phenylalanine and 3-methylhistidine metabolism of lambs fed rumen-protected leucine, KIC or IVA are presented in Table 9. Leucine used for protein synthesis (avg 4.42 umol/(kg*min)) tended to be decreased in the animals that were fed leucine ($p<.18$). Leucine from proteolysis and absorption (avg 4.66 umol/(kg*min)) was not altered due to dietary treatment. Leucine transamination to KIC (avg .83 umol/(kg*min)) was increased for the leucine-treated lambs ($p<.06$). KIC transamination to leucine (avg .62 umol/(kg*min)) and KIC oxidation (.22 umol/(kg*min)) were not affected by treatment. Phenylalanine flux (avg 1.29 uM/(kg*min)), tyrosine derived from phenylalanine (avg 51.8%) and 3-methylhistidine flux (avg 12.0 nmol/(kg*min)) were not altered due to treatment.

Correlations between protein metabolism measurements and growth, feed conversion and carcass parameters are included in Table 10. No significant relationships were noted between protein metabolism measurements and daily gain, muscle weight and fat depots. As phenylalanine conversion to tyrosine increased, feed to gain was worsened ($p<.05$).

Correlations between protein metabolism measurements and plasma metabolites are included in Table 11. No significant relationships were found between protein metabolism measurements and plasma leucine, KIC,

Table 9. The effect of feeding rumen-protected leucine (Leu), α-ketoisocaproate (KIC), or isovalerate (IVA) for approximately 100 days on lamb whole-body leucine, phenylalanine, and 3-methylhistidine metabolism.

	Control	Leu	KIC	IVA	SEM[b]	--Probability for > F[a]--- Overall Model	--Control vs--- Leu	KIC	IVA
Leu to protein synthesis, umol/(min*kg)	5.19	4.03	4.30	4.23	.58	.54	.18	.31	.27
Leu from proteolysis and absorption, umol/(min*kg)	5.34	4.35	4.57	4.39	.56	.58	.23	.65	.25
Leu to KIC umol/(min*kg)	.72	.97	.83	.82	.08	.30	.06	.62	.55
KIC to Leu umol/(min*kg)	.57	.66	.58	.67	.14	.99	.67	.97	.64
KIC oxidation, umol/(min*kg)	.15	.31	.26	.16	.09	.58	.26	.55	.99
Phe flux, umol/(min*kg)	1.27	1.34	1.29	1.28	.14	.99	.75	.94	.94
Tyr from Phe, %	47.9	56.7	52.4	50.0	8.2	.97	.53	.71	.85
3MH flux, nmol/(min*kg)	12.2	12.0	13.8	9.2	1.4	.21	.96	.53	.17

[a]Probability of > F for the overall ANOVA model, the contrast between the control vs Leu means, the contrast between the control vs KIC means, and the contrast between the control vs IVA means.

[b]SEM is pooled standard error of the mean.

Table 10. Correlations between leucine (Leu), phenylalanine (Phe) and 3-methylhistidine (3MH) flux measurements and growth, feed conversion and carcass composition measurements in growing lambs

	Gain	Feed /gain	Gastroc-nemius	Psoas	Longiss -imus	Back -fat	Perirenal fat
FOL[a]	-.08	.07	.10	-.02	-.38	.25	.34
FLO[b]	-.12	.13	.15	-.02	-.35	.25	.29
FAL[c]	-.23	.31	.07	-.29	-.16	.03	.06
FLA[d]	-.02	.01	-.13	-.18	-.32	.07	.30
FOA[e]	-.19	.28	.26	-.02	.30	-.07	-.38[x]
Phe flux	-.25	.21	.29	-.02	-.20	-.24	-.08
Phe to tyrosine	-.03	.40[*]	.08	.03	-.19	.03	-.02
3MH flux	-.13	-.23	.02	-.28	-.08	.35[x]	.24

[a]FOL = Leu to synthesis.
[b]FLO = Leu from proteolysis and absorption.
[c]FAL = Leu to KIC conversion.
[d]FLA = KIC to Leu conversion.
[e]FOA = KIC oxidation.
[x]$p<.10$, [*]$p<.05$.

Table 11. Correlations between leucine (Leu), phenylalanine (Phe) and 3-methylhistidine (3MH) flux measurements and plasma metabolite measurements in growing lambs

	Concentrations								
	Leu	KIC	Phe	Tyr[a]	3MH	Cort[b]	Ins[c]	Glgn[d]	I/G[e]
FOL[f]	-.02	-.13	-.48*	-.04	.03	-.08	.14	.33	.01
FLO[g]	.00	-.12	-.51*	-.06	.05	-.06	.07	.31	-.05
FAL[h]	.18	-.30	-.06	-.40	-.27	.29	.14	.14	.08
FLA[i]	.06	-.26	.00	-.18	-.27	.07	.41x	.26	.29
FOA[j]	.09	.09	-.06	-.10	.17	.18	-.45*	-.24	-.34
Phe flux	-.20	-.13	-.47*	-.20	-.09	.02	-.08	-.04	-.03
Phe to tyrosine	.23	-.06	-.38x	-.25	-.01	.06	.24	-.12	.29
3MH flux	-.03	-.15	.02	-.01	-.51	.06	.20	.32	.09

[a]Plasma tyrosine concentration.
[b]Plasma cortisol concentration.
[c]Plasma insulin concentration.
[d]Plasma glucagon concentration.
[e]Insulin to glucagon ratio.
[f]FOL = Leu to synthesis.
[g]FLO = Leu from proteolysis and absorption.
[h]FAL = Leu to KIC conversion.
[i]FLA = KIC to Leu conversion.
[j]FOA = KIC oxidation.
[x]$p<.10$, *$p<.05$.

Table 12. Correlations between leucine (Leu), phenylalanine (Phe) and 3-methylhistidine (3MH) flux measurements in growing lambs

	FOL[a]	FLO[b]	FAL[c]	FLA[d]	FAO[e]	Phe Flux	Phe to Tyr	3MH Flux
FOL[a]	1.00	.99****	.33	.46*	-.34	.13	.30	.12
FLO[b]		1.00	.33	.35	-.18	.14	.29	.12
FAL[c]			1.00	.72***	-.07	.17	.30	-.05
FLA[d]				1.00	-.74***	.12	.42x	-.04
FOA[e]					1.00	-.01	-.30	-.04
Phe flux						1.00	.54***	-.25
Phe to tyrosine							1.00	-.40x
3MH flux								1.00

[a]FOL = Leu to synthesis.
[b]FLO = Leu from proteolysis and absorption.
[c]FAL = Leu to KIC conversion.
[d]FLA = KIC to Leu conversion.
[e]FOA = KIC oxidation.
x p<.10; * p<.05; *** p<.0001; **** p<.0001.

tyrosine and 3-methylhistidine. As phenylalanine flux and phenylalanine conversion to tyrosine increased, plasma phenylalanine concentration decreased ($p<.05$ and $p<.10$, respectively). As the concentration of plasma insulin increased, KIC oxidation decreased and KIC to leucine conversion increased ($p<.05$ and $p<.10$, respectively).

Correlations between protein metabolism measurements are included in Table 12. As protein synthesis increased, leucine from proteolysis and absorption and KIC conversion to leucine were increased ($p<.0001$ and $p<.05$, respectively). A positive relationship was observed between KIC conversion to leucine and leucine conversion to KIC ($p<.0004$). Additionally, as KIC conversion to leucine increased, KIC oxidation decreased ($p<.0002$). As KIC conversion to leucine was increased, phenylalanine conversion to tyrosine was increased ($p<.06$). A positive relationship between phenylalanine flux and phenylalanine conversion to tyrosine was noted ($p<.005$), whereas a negative relationship between 3-methylhistidine flux and phenylalanine conversion to tyrosine was observed ($p<.06$).

Discussion

While gain and carcass parameters were similar to those reported in previous trials, the major finding in this study is that KIC did not appear to alter protein metabolism as measured by leucine, phenylalanine and 3-methylhistidine flux models. KIC did not increase leucine to whole-body protein synthesis nor decrease leucine from whole-body proteolysis as might be predicted from previous reports (Kirsch et al.,

1976; Chua et al., 1979; Poso et al., 1982; Tischler et al., 1982). Whole-body phenylalanine flux, a measurement of protein turnover independent of leucine metabolism, also was not altered by KIC treatment. Finally, muscle protein degradation, as measured by 3-methylhistidine flux, was not decreased by KIC.

There may be several reasons that an effect of KIC on protein metabolism was not detected. Firstly, the lambs in this study were over-night fasted, and it is possible that KIC may exert its effects on protein metabolism when animals are in the fed state. Animals in the fed state would have more substrates with which to increase muscle mass, and it is during the fed state that most of protein deposition takes place. Additionally, any effect KIC would have on increased insulin to glucagon ratios would be more dramatic during the fed period, when this ratio determines the amount of amino acids utilized for glucose production versus the amount utilized for protein synthesis.

Secondly, growth response to KIC has been greater in the early portion of each of the previous trials reported (Flakoll et al., 1988a, b). Net protein gain is greater during the early phase of lamb growth; therefore, the effect of KIC on protein metabolism may be greater during this phase. These studies measuring protein metabolism were conducted later on in the trials when the lambs were older and possibly less responsive to KIC.

Thirdly, the methods used to estimate protein metabolism may not have been specific to the organs that KIC affected. The leucine-KIC model estimates whole-body protein synthesis, proteolysis and

absorption, and oxidation; the phenylalanine model measures whole-body protein turnover; and the 3-methylhistidine model measures muscle (skeletal and gut) protein breakdown. If muscle protein synthesis was increased without significantly increasing whole-body protein synthesis, these methods would not detect a change. For example, KIC could be directing protein from gut and liver tissues to muscle tissue. This partitioning of nutrients needs closer examination.

Parameters of leucine kinetics in this trial are in agreement with those in previously reported studies (Grisdale, 1986; Nissen and Ostaszewski, 1985; Davis et al., 1981; Pell et al., 1986). In the present experiment, only 16% of the leucine flux was transaminated to KIC and only 11% of the leucine produced was the result of transamination from KIC. Other reports of transamination in lambs have shown leucine conversion to KIC to be 18% of the total leucine flux (Nissen and Ostaszewski, 1985). Conversely, reports with nonruminants have demonstrated leucine to KIC conversion to be 59% in humans (Matthews et al., 1981), 62% in the pig (Helland et al., 1986) and 82% in the dog (Nissen and Haymond, 1981). Clearly, this is a distinct difference between ruminants and nonruminants.

Because of their decreased transaminase activity, ruminants may be more responsive than nonruminants to dietary inputs of leucine in terms of plasma leucine concentration. Leucine-treatment tended to decrease gain in lambs, which appeared to be through decreased feed intake. Plasma ratios of leucine to phenylalanine or tyrosine were 20% greater in the leucine-treated lambs than in the controls and 33% greater than

the KIC-treated lambs. This imbalance of leucine to phenyalanine and tyrosine may be the reason for depressed feed intake.

Although there are no previous reports of phenylalanine metabolism in ruminants, Moldawer et al. (1983) reported pheylalanine flux in postabsorptive rats to be 6.9 umol/(kg*min), which is considerably greater than the results reported in the present experiment. The leucine flux they reported (10.9 umol/(kg*min) was also higher than the present reports. However, they also found significant conversion of phenyalanine to tyrosine. They estimated that 54% of tyrosine oxidized was derived from phenylalanine, which would be in close agreement with the present data in lambs. These data present evidence confirming that estimates of protein turnover by tyrosine needs to include contributions from phenylalanine flux.

There are no known previous reports of plasma 3-methylhistidine flux in lambs. In previous work where 3-methylhistidine metabolism was examined in lambs (Harris and Milne, 1980), it was found that 3-methylhistidine transfer from blood to urine was slow compared with that in humans (Long et al., 1975), rats (Young et al., 1972), rabbits (Harris et al., 1977) or cattle (Harris and Milne, 1981). Less than 50% of a ^{14}C labelled 3-methylhistidine was recovered from lamb urine after 7 days. In contrast, ^{14}C-labelled 3-methylhistidine was almost quantitatively collected in the urine of humans, rats, rabbits and cattle within 2 to 7 days. This difference is thought to be due to the large 3-methylhistidine pools within the lamb body in comparison to urinary output and makes analysis of urinary 3-methylhistidine in lambs

an unacceptable method for estimation of muscle protein breakdown. Turnover of the plasma pool of 3-methylhistidine may, however, provide as alternative method of measuring muscle protein breakdown. Further studies are necessary to validate this methodology in lambs.

Although correlation coefficients calculated in this experiment indicate only relationships and not causes and effects, several conclusions can be drawn. When comparing the measurements of protein metabolism with measurements of growth, feed conversion and carcass composition, it is of note that very few significant correlations were found. This suggests that the prediction of long-term parameters is difficult with protein metabolism measurements made during short-term infusion studies. It also stresses the importance of when these measurements are taken.

The only significant relationship found between performance measurements and protein metabolism measurements was that phenylalanine to tyrosine conversion was positively correlated with feed conversion, meaning as more phenylalanine was converted to tyrosine and feed conversion tended to be worsened. An additional observation was that, as the conversion of phenylalanine to tyrosine increased, phenylalanine flux was increased and plasma phenylalanine concentration was decreased. The reasons for this are unclear.

When comparing the various parameters of the leucine model, increasing the interconversion of leucine to KIC and KIC to leucine is positively correlated with KIC oxidation. This would stand to reason as it has been demonstrated that feeding high protein diets (Wohlhueter and

Harper, 1970), leucine (Wohlhueter and Harper, 1970) or KIC (Khatra et al., 1977) to rats increases branched-chain amino acid dehydrogenase activity. Additionally, in the present study protein synthesis was positively correlated with both proteolysis/absorption and the amount of KIC converted to leucine, suggesting that increased leucine entry may increase protein synthetic rate.

Few of the measurements between leucine, phenylalanine and 3-methylhistidine models were significantly correlated. The only trends were that KIC conversion to leucine tended to be positively correlated with phenylalanine to tyrosine conversion, whereas 3-methylhistidine flux tended to be negatively correlated with phenylalanine to tyrosine conversion. The significance of these relationships is unclear, but the apparent lack of significant relationships between the three models raise questions regarding the sensitivity of such models and emphasizes the caution which must be taken when interpreting data from a single amino acid flux model.

In conclusion, KIC and its metabolites, leucine and IVA, did not alter protein metabolism in overnight-fasted lambs as measured by leucine, phenylalanine or 3-methylhistidine flux models suggesting that KIC does not increase growth and carcass composition by sparing protein of lambs in the nonfed state.

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GENERAL DISCUSSION

The major finding from these experiments is that α-ketoisocaproate (KIC) improves gain and feed conversion in cattle and lambs. Additionally, external and internal fat depots were decreased, psoas, gastrocnemius and longissimus muscle weights were increased, and humerus and tibia bone weights were increased.

When considering the possible mechanisms for KIC's action, foremost consideration must be given to KIC's proposed effects of increasing protein synthesis and decreasing proteolysis. These effects would explain improved gain and feed conversion, increased muscle mass and decreased fat deposition. However, when three independent models measuring protein metabolism were used on KIC-treated lambs, no improvement in whole-body protein synthesis, whole-body proteolysis and muscle proteolysis were detected.

It is not possible for muscle mass to be increased without in some way altering protein metabolism. It needs to be kept in mind, however, that the protein turnover measurements were made during two hours of one day out of the approximately 100 days of treatment. This fact allows for several possible explanations as to how muscle mass could be increased, although protein metabolism measurements were not altered.

Firstly, most protein deposition in an animal occurs during and shortly after a meal. Additionally, much of the energy required for protein turnover is spent during this same period. The reported protein metabolism studies were conducted on lambs after an over-night fast. If

protein deposition is increased by KIC during or shortly after a meal, it would not be detected by these experiments.

Additionally, it was demonstrated, in the experiments in which it was measured, that KIC increased the ratio of insulin to glucagon. Any effect KIC would have on increased insulin to glucagon ratios would be most dramatic during the fed period when this ratio determines the amount of amino acids utilized for glucose production versus the amount utilized for protein synthesis. Increased insulin to glucagon would direct more amino acids to protein synthesis and result in fewer being catabolized. Therefore, further research examining the effects of KIC on ruminant protein metabolism during a fed state would certainly be warranted.

Secondly, during the earlier growth phase of a ruminant, a greater portion of body weight gain is the result of muscle gain, as opposed to fat or bone gain, than in the later growth phase. Therefore, net protein deposition is greater during this early phase of growth. If net protein deposition is increased more dramatically in the early portion of the lamb growth phase, the effect of KIC on protein metabolism and deposition would be expected to be greater during this period. Protein metabolism studies reported here were executed in the later portion of the growth phase and would not detect any such alterations. Growth, however, was measured at weekly or bi-weekly intervals during the reported experiments and was found to be increased more dramatically during the early portion of each experiment reported.

Thirdly, it could be postulated that KIC increased net protein

deposition by suppressing stress related catabolism. Again, most of the chronic stress in these trials occurred early in each experiment when animals were adjusting to new environments, pathogens, different feed sources and human contact through feeding and handling. Protein metabolism was not measured during this period. The demonstrated growth-response to KIC early in each trial would also support this theory. Additionally, concurrent studies indicated that lymphocyte blastogenesis and antibody production, which are normally depressed during stress, were increased by KIC treatment. It is possible that the possibility of KIC moderating the physiological results of stress has been overlooked in the clinical trials in which KIC was used under stressful conditions such as septis, renal or hepatic failure and starvation. Further work examining the effect of KIC on protein turnover during stress is necessary.

Finally, the methods used to estimate protein metabolism may not have been specific to the area that KIC affected. The leucine-KIC model estimates whole-body protein synthesis, proteolysis and absorption, and oxidation; the phenylalanine model measures whole-body protein turnover; and the 3-methylhistidine model measures muscle (skeletal and gut) protein breakdown. If muscle protein synthesis was increased without significantly increasing whole-body protein synthesis, these methods would not detect a change. For example, KIC could be directing protein from gut and liver tissues to muscle tissue. Studies examining the effects of KIC on protein metabolism of various ruminant tissues in vivo, tracing the flow of nutrients to the various organs during KIC

treatment, is necessary to fully understand the mechanism of KIC action.

It has been argued that selected amino acid tracer studies to estimate protein metabolism may not correlate well with actual nitrogen balance due to methodological problems. Although whole-body nitrogen balance was not measured, this was also suggested in the present studies. If this is the case then the disparity between growth and muscle weights, and protein metabolsim studies are easily explained. Clearly, nitrogen balance studies with growing ruminants would help explain the effects of KIC on protein metabolism.

Another possible mechanism that KIC may improve growth could be through an improvement of immune function. Improved immune function could translate into fewer sub-par performing animals and an overall increase in average performance.

It was also apparent from the reported experiments that other metabolites of KIC, leucine and isovalerate did not produce similar responses as did KIC. Therefore, either the physiology of leucine and IVA metabolism do not allow them to produce effects similar to KIC, or it does not appear that KIC exerts its effects through these metabolites.

Because KIC is metabolized mainly by gut and liver tissues, it would follow that gut and liver tissues would be the main location of KIC action. Several speculations about the mechanism of KIC in the gut and liver can be made. First, the splachnic area has the greatest rate of protein turnover in the body; therefore, KIC could affect growth and muscle deposition by lowering gut and liver protein turnover. Second,

KIC could affect cholesterol and ultimately steroid hormone metabolism through its catabolism to β-hydroxy-β-methylglutaryl CoA or its oxidation to β-hydroxy-β-methylbutyrate; the liver is the major tissue that produces cholesterol. KIC could also affect liver cholesterol metabolism by altering cholesterol transport protein production, because it has been demonstrated that KIC increased another liver transport protein, albumin. KIC could alter gut and liver cell metabolism directly, possibly resulting in a sparing of nutrients crossing the splanchnic bed. Additionally, the gut produces several hormones, and KIC could alter any of these. The possible interactions of KIC with gut and liver tissues are immense and definitely require further examination.

It could be argued that the growth response to KIC may be through a ruminal effect on microorganisms as has been demonstrated with branched-chain fatty acids. However, data from the reported experiments would indicate that the effect of KIC is postruminal. First of all, KIC was effective when delivered intraperitoneally. In addition, oral delivery was more effective when it was protected from ruminal degradtion.

KIC produced similar resposes to gain in lambs when delivered orally at 15 g/d (1% of intake) unprotected from ruminal degradation, intraperitoneally at 3 g/d (~.2% of intake), and orally protected from ruminal degradation at .75 g/d (.05% of intake). Other dosage levels in lambs were not tested. In the only dose-response study conducted, KIC produced a better gain response in cattle when fed at 1.8 g/d (.02% of intake) than at 6.4 (.07% of intake) or 18.0 g/d (.20% of intake).

This optimal response was greater than for the lambs fed ruminally-protected KIC. On a body-weight basis, the optimal dose for the cattle fed KIC was almost four-fold greater than the dose for the lambs fed ruminally-protected KIC (4 versus 15 mg KIC/kg body weight). The dosage delivered to the lambs, therefore, may have been higher than optimum. The optimal amount of KIC under various conditions is an area that will require considerable research.

In each experiment conducted, KIC was administered with a basal diet relatively high in protein and energy. The response KIC would evoke if administered to animals on a lower plane of nutrition will need to be addressed in further research. If KIC spares protein and (or) energy, it may require less nutrient input to obtain equivalent gains.

Internal and external fat depots consistently were decreased in the animals administered KIC. Additionally, milk fat production in lactating ruminants has been demonstrated to be increased. There are several reasons that could be postulated for these observations. The first involves KIC's proposed decrease of protein turnover. In the growing ruminant, the energy that would be saved by a decrease in protein turnover would be directed to increased protein muscle synthesis. However, in the lactating ruminant, it appears that these energy savings would be directed to the mammary gland. A second possibility is if KIC treatment would increase lipolysis or suppress lipogenesis in adipose tissue increased muscle growth and decreased fat depots would be predicted. Finally, KIC is directly linked to fat metabolism through its conversion to ketone bodies and

β-hydroxy-β-methylglutaryl Co A, a precursor for cholesterol and steroid hormones. Ketone bodies, however, have been shown to suppress lipogenesis. Therefore, no clear mechanism for KIC to act on fat metabolism can be drawn from the present studies.

In conclusion, while the mechanism(s) is not entirely clear, KIC improves cattle and sheep growth and efficiency, decreases fat and increases muscle weight. These improvements certainly have the potential for economic improvement by the ruminant producer. However, there is much yet to be learned about the physiological metabolism of KIC, which may allow researchers to further improve the efficiency of ruminant production.

ACKNOWLEDGEMENTS

Although a Ph.D. program requires dedication and work by the candidate, it cannot be completed without support. Most sincere appreciation is extended to Dr. Steve Nissen for providing the opportunity for doing this work and especially for his guidance, assistance, encouragement and friendship. Gratitude is expressed to Dr. Allen Trenkle for promoting my interest in research and protein metabolism, and for maintaining this interest over the years in and out of the classroom setting.

I want to thank Don Beitz for stimulating discussions, providing valuable advice, and serving on my graduate committee. Appreciation is also extended to Dr. Ted Huiatt and Dr. Richard Seagrave for serving on my graduate committee.

Appreciation is expressed to my fellow graduate students, particularly my collaborator Mike VandeHaar, whom I enjoyed working with, and also Gail Kuhlman, Kevin Hannon, and Ann Gronowski for scientific and not-so-scientific conversations providing an enjoyable environment. Much appreciation is also extended to the numerous technicians and animals caretakers that helped with this work and also provided a pleasant environment and stories for years to come.

Special thanks are extended to my loving parents, Alden and Wilma; my brother, Tim; and my sister, Joan for their continued encouragement throughout my seemingly endless years of higher education. My two sons, Michael and Andrew, have taught me a great deal about life and deserve much credit for helping me to maintain a clear perspective on

what is important in life.

Finally, foremost appreciation is extended to my wife, Candace, for her love, encouragement, assistance, and cooperation, without which the opportunity for graduate study would have only been a dream.

APPENDIX A

Short-term Intravenous Administration of α-ketoisocaproate (KIC) to Lambs

In a preliminary experiment, ewes weighing 29 kg were injected intravenously via indwelling jugular catheters for 21 days with saline or sodium-KIC. Six lambs were injected daily with 25 ml saline, 4 lambs with 1 g of Na-KIC in 25 ml saline and 4 lambs with 2 g of Na-KIC in 25 ml saline. After injection, 5 ml of heparenized saline was injected to flush the cathetors. The lambs were housed together in one group, but were fed individually a corn-soybean meal based diet (Table 1). Feed was given twice daily with continual access to water. Body weight gain and feed intake measurements were made weekly. Wool growth was measured by clipping a known marked area on each side of each lamb at both the start and at the end of the experiment. Statistical analysis was made by analysis of variance and comparing the KIC treatment means with the control mean via t-test.

Lamb growth and feed conversion are presented in Table 2. Daily intravenous injections of 1 or 2 g of KIC increased gain 14% ($p<.09$) and 29% ($p<.01$), respectively, above the saline treated animals. Neither daily feed intake nor feed conversion were altered by intravenous KIC treatment ($p<.12$ and $p<.15$, respectively). Wool gain was not significantly altered by KIC.

Table 1. Composition of basal diet fed lambs infused with daily intravenous doses of α-ketoisocaproate (KIC)

Ingredient	% of total mix
Corn grain	48.70
Dehydrated alfalfa[a]	25.00
Expeller soybean meal[b]	19.00
Molasses, sugarcane	5.00
Corn oil[c]	1.00
Limestone	.74
Salt	.50
Vitamin A premix[d]	.05
Trace mineral premix[e]	.01
Calculated:	
Net energy-maintenance, Mcal[f]	1.82
Net energy-gain, Mcal[f]	1.18
Metabolizable protein, %[g]	9.7
Leucine, %[f]	1.85

[a]20% crude protein.
[b]Soy-Plus, West Central Cooperative, Ralston, IA.
[c]Mazola, CPC International Inc., Cedar Rapids, IA.
[d]5,200,000 IU vitamin A/kg, Hoffmann-LaRoche, Inc., Nutley, NJ.
[e]Ca, 13-15%; Zn 12%; Mn 8.0%; Fe 10%; Cu 1.5%, I .2%; Co .1%, Calcium Carbonate division of J. M. Huber Corporation, Quincy, IL.
[f]Calculated from National Research Council values (National Research Council, 1982).
[g]Calculated from Burroughs et al. (1974).

Table 2. The effect of daily intravenous infusion of α-ketoisocaproate (KIC) for 21 days on lamb growth, feed conversion, and wool growth

	----NaKIC Infused Daily, g----			
Parameter	0	1	2	SEM[a]
n	4	4	5	
Daily gain, g	209	244^{+}	265**	13
Daily intake, g	1034	1161^{x}	1143	52
Feed/gain	5.0	4.8	4.3^{x}	.3
Fat-free wool gain, mg/(cm^2·d)	5.4	4.5	4.5	.5

[a]SEM is pooled standard error of the mean.

^{x}P<.15, $^{+}$P<.10 and **P<.01 that treatment mean are not different from the control.

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APPENDIX B

Liver Lipid Concentration and Composition of Lambs Receiving Daily Intraperitoneal Injections of α-ketoisocaproate (KIC)

Liver samples were removed from lambs receiving daily intraperitoneal injections of KIC (Exp. 1, Section I). Liver lipids were extracted by chloroform-methanol extraction (2:1, v:v; Rotenberg and Christensen, 1976). Extracts were dried and total lipid weight recorded. The lipid extracts were resuspended in isopropanol for enzymatic analysis of total cholesterol (Centrifichem, Baker Instruments Corporation, Allentown, PA). Fatty acid composition of the liver lipid extract was determined by gas chromatography (Varian 3700G) of the fatty acid methyl esters (Arrendale et al., 1983).

The effects of intraperitoneal administration of KIC on lamb liver composition is reported in Table 1. Liver fat and cholesterol were reduced by 21% ($p<.11$) and by 25% ($p<.05$) due to KIC treatment. Liver C16, C17, and C18 fatty acids were lowered with KIC treatment by 27% ($p<.06$), 41% ($p<.01$) and 24% ($p<.08$), respectively. Total unsaturated fatty acids (C16:1, C18:1, C18:2, and C18:3) were not significantly decreased by KIC treatment, whereas total saturated fatty acids were decreased 26% ($p<.05$).

The ruminant obtains fatty acids from two sources: dietary absorbtion or de novo synthesis. Due to hydrogenation by microorganisms in the rumen, fatty acids absorbed from the gut and presented to the liver via the portal system are mainly saturated. Therefore, steric acid (16:0) is the major fatty acid supplied by the diet. The primary

precurser for fatty acids synthesis by the ruminant is extramitochondrial acetyl CoA and not glucose. Minimal fatty acid synthesis occurs in the ruminant liver due to the low activity of hepatic citrate lyase, whereas adipose tissue is responsible for approximately 90% of fatty acid synthesis in ruminants. De novo synthesized fatty acids are readily desaturated (25 to 75% of total 18:0 synthesized is desaturated) in preference to those of exogenous origin. Therefore, as fatty acid synthesis is increased, desaturase activity is increased.

Liver has minimal fatty acid synthetase and desaturase activities. However, the liver is the major site of fatty acid removal (25% of total fatty acids). Liver uptake of fatty acids is a function of total plasma fatty acid concentration. Shorter chain and desaturated fatty acids are taken up by the liver faster than longer chain and saturated fatty acids.

In the present experiment, liver saturated fatty acids were reduced while unsaturated fatty acids were not altered by KIC treatment. The ratio of saturated to unsaturated fatty acids could be lowered by 1) increasing the adipose desaturase activity, which usually means an increase in de novo synthesis, 2) increasing the preference of the liver for unsaturated fatty acid uptake, and 3) increasing lipolysis. Plasma fatty acid content increased in Exp. 2 and liver lipid and cholesterol content were decreased in the Exp. 1, suggesting that an increase in lypolysis may be likely. Further research is required to specifically define the effect of KIC on lipolysis and lipogenesis.

Table 1. Effects of daily intraperitoneal injections of α-ketoisocaproate (KIC) for approximately 120 days on lamb liver lipid composition

Parameter	Saline	KIC	SEM[a]	P<[b]
Liver lipid, %	4.09	3.18	.38	.11
Liver cholesterol, %	.264	.197	.023	.05
Liver fatty acids, %				
C16	.7127	.5180	.0690	.07
C16:1	.1167	.1010	.0156	.50
C17	.1077	.0639	.0111	.01
C18	.9726	.7369	.0893	.08
C18:1	1.2213	.9567	.1263	.15
C18:2	.9042	.7578	.0936	.28
C18:3	.0501	.0462	.0058	.64
Total saturated	1.7931	1.3188	.1585	.05
Total unsaturated	2.2923	1.8617	.2324	.21
Saturated/unsaturated	.788	.719	.034	.16

[a]Pooled standard error of the mean.

[b]Probability that treatment means are not different.

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APPENDIX C

Small Intestines, Mesenteric Fat, Lymph Nodes, and Separable Carcass Fat, Lean and Bone from Lambs Fed α-ketoisocaproate (KIC), Leucine (Leu) and Isovalerate (IVA; Exp. 2, Trial 2, Section 2)

Small intestine, mesenteric fat, lymph nodes, and separable carcass fat, lean, and bone were sampled in Trial 2 of Exp. 2 in Section 2. Small intestines was removed from the lambs at termination, stripped of digesta, and weighed. Mesenteric fat was dissected from intestinal and stomach tissue and weighed. Right and left cervical and illiac lymph nodes were dissected from frozen carcasses and weighed. Carcasses were physically separated into fat, lean, and bone tissue and weighed. Results are included in Table 1.

Table 1. The effect of feeding rumen-protected leucine (Leu), α-ketoisocaproate (KIC), or isovalerate (IVA) for approximately 100 days on weight of lamb small intestines, mesenteric fat, lymph nodes and carcass fat, lean and bone

	Control	Leu	KIC	IVA	SEM[a]	P>F[b]	P>F[c]
Small intestine, %	0.78	0.85	0.78	0.81	.11	.25	.98
Mesenteric fat, %	3.48	3.92	3.71	3.93	.49	.99	.53
Lymph nodes, % x 1000:							
Right cervical	10.8	14.8	11.7	11.1	1.7	.34[d]	.71
Left cervical	9.9	14.9	13.1	11.1	1.5	.14[e]	.17
Right ileac	3.1	3.2	3.2	2.7	.4	.71	.84
Left ileac	2.8	3.8	2.6	2.5	.4	.09[f]	.67
Carcass, %							
Fat	27.1	26.8	28.6	29.6	1.3	.56	.55
Lean	53.1	54.5	52.6	54.5	1.0	.51	.76
Bone	19.8	18.7	18.8	18.1	.7	.31	.32

[a]SEM is pooled standard error of the mean.

[b]Probability of > F for overall model.

[c]Probability of > F for contrast between control and KIC means.

[d]Probability of > F for contrast between control and leucine means = .11.

[e]Probability of > F for contrast between control and leucine means = .03.

[f]Probability of > F for contrast between control and leucine means = .08.

APPENDIX D

Summary of Observations Concerning Gas Chromatography/Mass Spectroscopy (GC/MS) Method for 3-Methylhistidine (3MH)

1. Acetonitrile is a better solvent than pyridine.

2. Moderate heat (~70 C. for 1 hr) after derivatization increases abundance.

3. Butylsilyltrifluroacetamide (BSTFA) results in greater abundance than tributyldimethylsilane (TBDMS) monitoring BSTFA @ 196 AMU and TBDMS @ 340 AMU.

4. There were no changes in abundance after lengthening or shortening purge time.

5. There were no changes in abundance when injector temperature was lowered from 250 to 150° C.

6. No 3MH was detected in keto rinse from the cation column.

7. With 2 ml of cation resin (50%), 25% NH_4OH is strong enough to elute 3MH (25 to 100 % was tested).

8. Decreasing the oven temperature to 50° C initially, while maintaining the injector temperature at 250° C dramatically increased abundance.

9. Siliconizing glass dram vials appeared to improve abundance very little (however, it may help older vials).

10. Increasing the amount of 3MH standard resulted in a linear (corr ~.98) increase in abundance.

11. Increasing the amount of plasma resulted in a linear (corr ~.97) increase in abundance.

12. Differing ratios of d-3 3MH and 3MH stds with or without 1 ml of sheep plasma (2,0;1.5,.5;1,1;.5,1.5;0,2 or 1,0;.75,.25;.5,.5;.25,.75;0,1) resulted in linear curves.

13. TBDMS without 1% TBDMClS catalyst is a better dervative than BSTFA, as it consistently produces abundances greater than or equal to BSTFA, and it is far more stable.

14. HCl gassing of dried sample before derivatization resulted it slightly greater abundances, especially with BSTFA.

15. Heating samples to 105^{o} C for 15 to 20 min. after drying greatly improves the abundance yields.

16. Acetone and 1.5 N perchlorate protein precipitation appear to be equivalent.

APPENDIX E

Outline of 3-Methylhistidine Analysis

1. Add sample (2 ml if plasma) + 177 nmol 1-methylhistidine to 10 ml plastic tube.

2. Deproteinize with 3.0 ml 1.5 N $HClO_4$ per ml sample.

3. Vortex and centrifuge @ 3000 for 15 min.

4. Prepare cation columns with 2 ml Dowex resin (50%). Wash with 4 ml .01 N HCl.

5. Pour sample over column. Rinse with 3 ml of 0.01 N HCl. Discard rinse.

6. Elute aminos with 4 ml 25% NH_4OH. Collect in very clean dram vials.

7. Dry at 55° C. under nitrogen. When dry increase temperature to 105° C for 15 to 20 min.

8. Cool. Add 100 ul acetonitrile (Regis Chemical Co., Morton, IL) and 100 ul TBDMS without TBDMCIS (Regis Chemical Co., Morton, IL). Cap tighlty with TEFLON-lined caps.

9. Vortex thoroughly and place in 80 C. oven for 1 hour.

10. Label plastic conical autosampler vials and place in same oven for 1 hr to dry.

11. Remove samples and vials from oven, cool, and transfer samples from dram vials to autosampler vials, cap, and place in autosampler rack.

12. Inject into GC/MS (Hewlett-Packard 5890/5790a) using DATA:MHSIMB.M, which monitors 238.1, 239.1, 240.1, and 241.1 AMU with

injector and detector @ 285 C., and oven initially at 50 C. Oven temperature initial temperature at 50° C, ramp 1 at 40° C/min to 220°, hold for 5 min., and ramp 2 at 40°/min to 300° C, hold for 1.3 min. Retention time is roughly 7.8 min. Column is 25 m x .22 mm i. d. x .11 um film thickness, crosslinked silicone gum phase capillary column (HP-1, Hewlett-Packard, Avondale, PN).

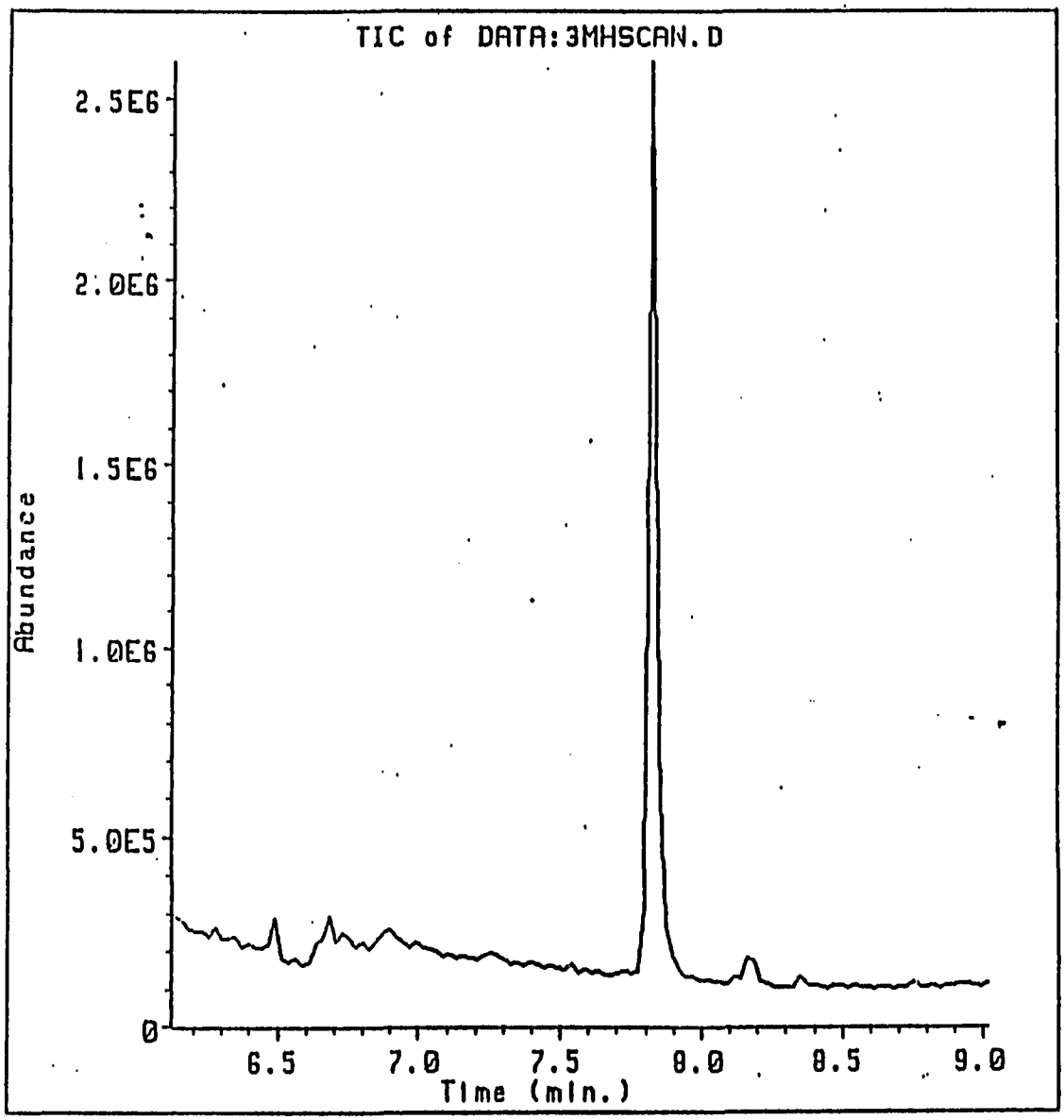

Figure 1. Total ion chromatograph (TIC) of 3-methylhistidine (retention time = 7.8 min) on a Hewlett-Packard 5790a mass selective detector under the conditions described in the outline above

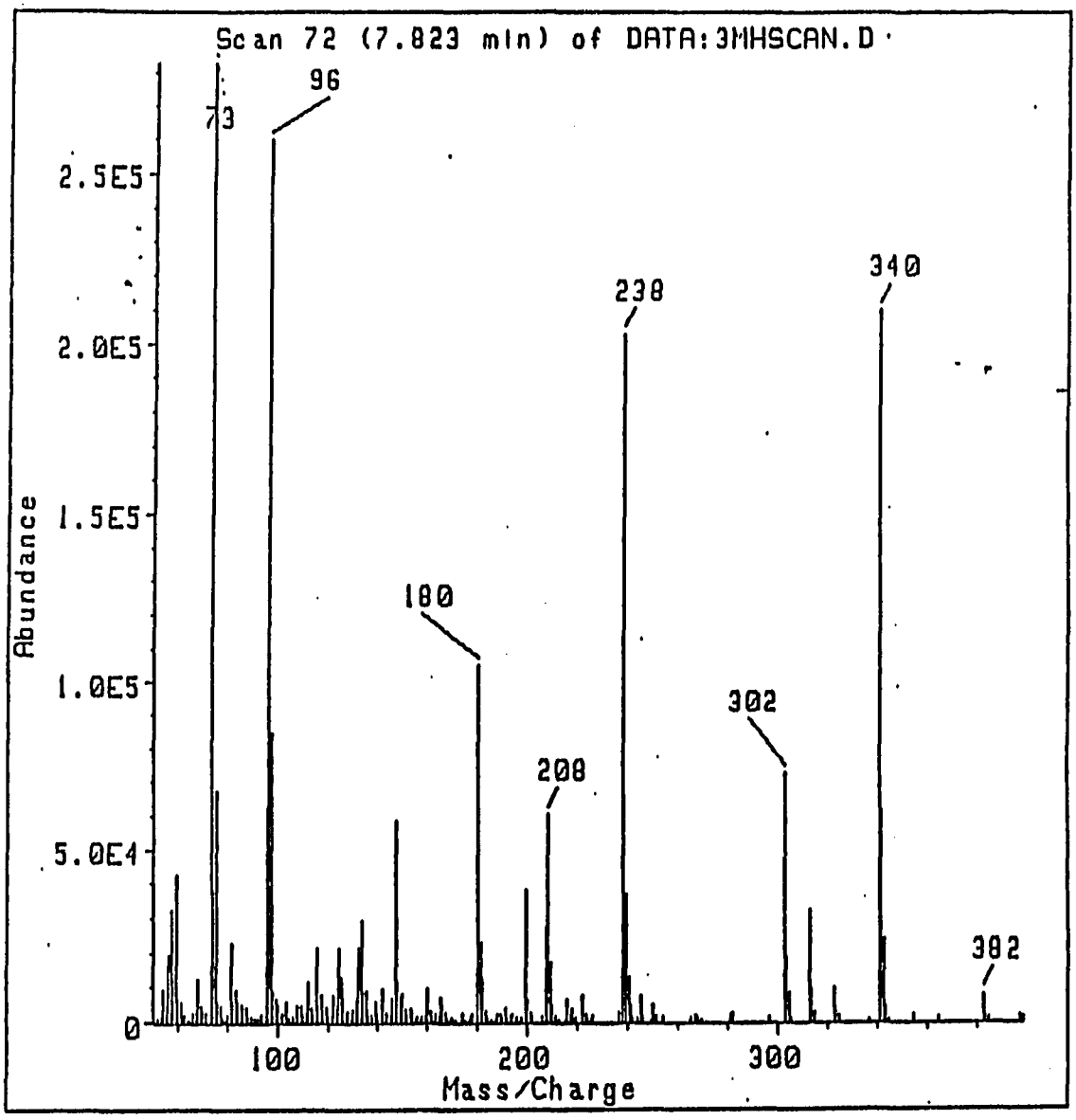

Figure 2. Spectral scan of 3-methylhistidine (retention time = 7.8 min) on a Hewlett-Packard 5790a mass selective detector under the conditions described in the outline above

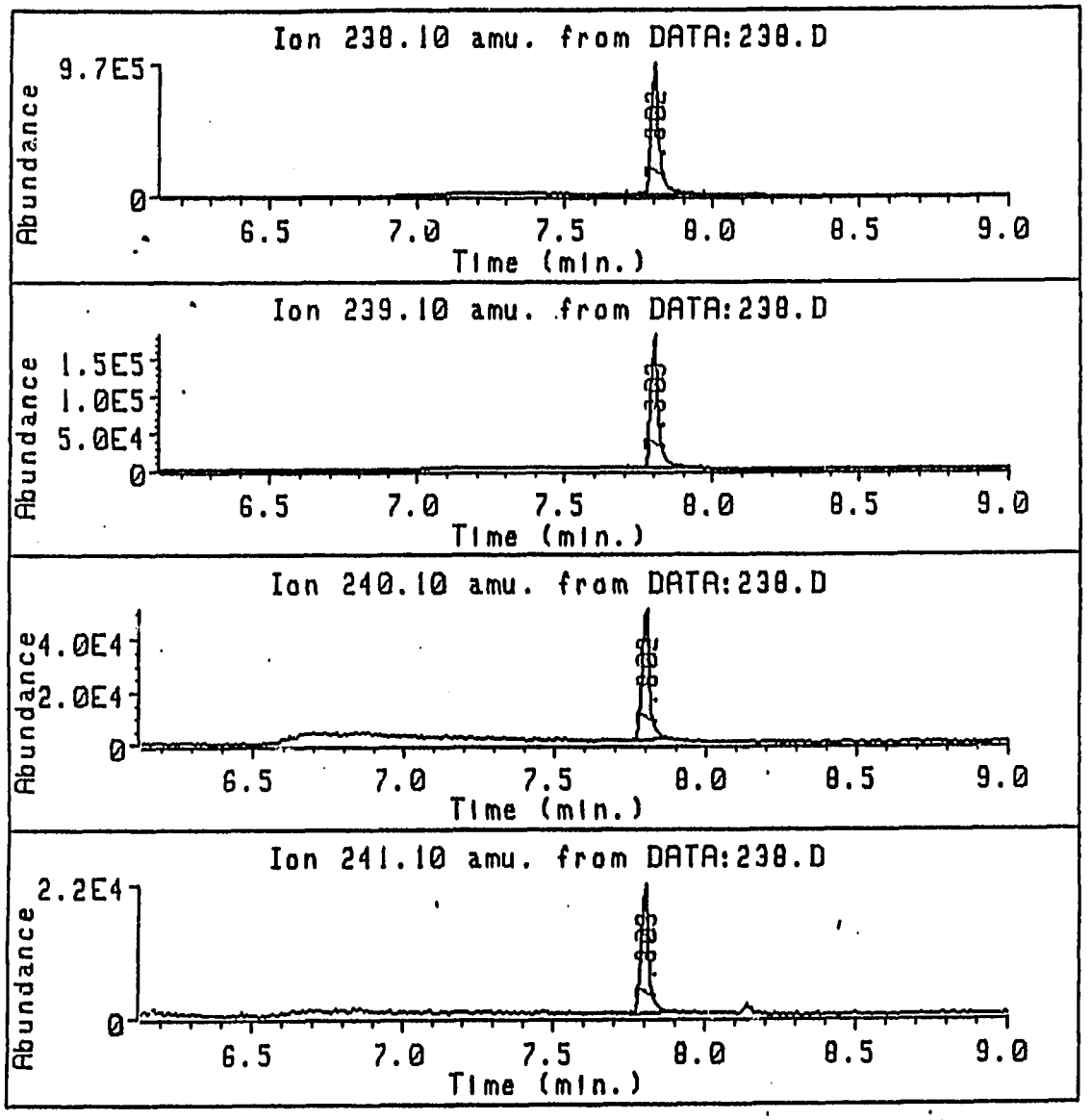

Figure 3. Selected ion monitoring (SIM) chromatographs of 3-methylhistidine with a Hewlett-Packard 5790a mass selective detector monitoring for masses 238.1, 239.1, 240.1 and 241.1 AMU as described in the outline above

*** Height Percent ***

Report by Retention Time

Operator: 27 Nov 87 10:01 pm
Sample Info :
Misc Info:
Integration File Name : DATA:238.I
Method Index : 3 Bottle Number : 1

Ret Time	Signal Descr	Type	Area	Height	% Pk	% Sg	% LPk	% LSg
7.803	Mass 241.10 amu	BB	363385	21485	1.74	100.00	2.19	100.00
7.802	Mass 240.10 amu	BB	866695	50798	4.11	100.00	5.18	100.00
7.803	Mass 239.10 amu	BB	3264269	182921	14.80	100.00	18.65	100.00
7.802	Mass 238.10 amu	BV	17825410	980912	79.35	100.00	100.00	100.00

Figure 4. Report from chromatograph in Figure 3